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Inhibitory effects of astragalin on lipopolysaccharide-induced inflammatory response in mouse mammary epithelial cells

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ABSTRACT

Background: Tea brewed from the leaves of *persimmon* or *Rosa agrestis* have several medical functions including treating allergy, antiatopic dermatitis, and anti-inflammatory effects. The objective of this study was to investigate the molecular mechanisms of astragalin, a main flavonoid component isolated from these herbs, in modifying lipopolysaccharide (LPS)-induced signaling pathways in primary cultured mouse mammary epithelial cells (mMECs).

Materials and methods: The mMECs were treated with LPS in the absence or presence of different concentrations of astragalin. The expression of proinflammatory cytokines tumor necrosis factor α, and interleukin 6, as well as nitric oxide production were determined by enzyme-linked immunosorbent assay and Griess reaction, respectively. Cyclooxygenase-2, inducible nitric oxide synthase, toll-like receptor 4 (TLR4), nuclear factor-κB (NF-κB), inhibitor protein of NF-κB (IκBα), P38, extracellular signal-regulated kinase, and c-Jun N-terminal kinase were measured by Western blot.

Results: The results showed that astragalin suppressed the expression of tumor necrosis factor α, interleukin 6, and nitric oxide in a dose-dependent manner in mMECs. Western blot results showed that the expression of inducible nitric oxide synthase and cyclooxygenase-2 was inhibited by astragalin. Besides, astragalin efficiently decreased LPS-induced TLR4 expression, NF-κB activation, IκBα degradation, and the phosphorylation of p38, extracellular signal-regulated kinase in BMECs.

Conclusions: Our results indicated that astragalin exerts anti-inflammatory properties possibly via the inactivation of TLR4-mediated NF-κB and mitogen-activated protein kinases signaling pathways in LPS-stimulated mMECs. Thus, astragalin may be a potential therapeutic agent for bovine mastitis.

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1. Introduction

Mastitis is defined as infection and inflammation of the mammary gland [1]. It not only poses a threat to women health but also to dairy cows, resulting in great economic loses in dairy cattle industries. Bovine mastitis is caused by a wide array of microorganisms, including Gram-positive pathogen such as *Staphylococcus aureus* (*S aureus*) and Gram-negative pathogen such as *Escherichia coli* (*E coli*) [2]. These bacteria can invade the mammary gland by penetration through the teat canal and lead to mammary gland inflammation [3]. Lipopolysaccharides (LPS) or endotoxin, which is possessed by *E coli* in the outer membrane of bacterial cell wall, is identified as a vital virulence factor of mastitis [4]. Toll-like receptors (TLRs), which are differentially expressed on myeloid lineage cells, such as dendritic cells, monocytes or macrophages and nonimmune cells, including endothelial cells, fibroblasts, adipocytes, and epithelial cells, are critical pattern recognition receptors that localized as the first line of innate defense by recognizing pathogen-associated molecular patterns (PAMPs) leading to innate and adaptive immune response [5,6]. Up to now, there are 13 mammalian TLRs that have been reported [7]. The expression of TLR4 on the cell surface can be activated by several bacterial surface molecules, including the conserved LPS of Gram-negative bacteria. LPS-activated TLR4/NF-κB triggers the formation of signaling complexes and leads to the release of various pro-inflammatory cytokines and mediators such as tumor necrosis factor α (TNF-α), interleukin (IL)-6, and IL-1β [8]. Previous reports have shown that inducible nitric oxide synthase (iNOS) and Cyclooxygenase-2 (COX-2) were also upregulated by binding of LPS or TNF-α in mammary epithelial cells and further aggravating the inflammatory immune response during infection [9–11].

When an intramammary *E coli* infection occurs, bacteria that enter the mammary gland release LPS. As a reaction, a host immune response is initiated where the polymorphonuclear neutrophil leukocytes (PMNs) play a pivotal role in clearing the mammary gland by phagocytizing invading bacteria [12]. Besides, other cell types in the udder, such as mammary alveolar epithelial cells also come in direct contact with the bacteria invasion and act as more than a physical barrier for pathogens, but also participate in the immune regulation during mastitis via producing inflammatory mediators [6,13]. Therefore, it is of importance to investigate the inflammatory and immune response in mammary epithelial cells during LPS infection.

Nowadays, despite various therapeutic strategies have been developed to prevent mastitis, there are still no reliable and effective therapies against the disease [14]. In the meantime, many researchers reported that many compounds that extracted from natural plants exerting beneficial effects against mastitis both *in vitro* and *in vivo*. Astragalin (shown in Fig. 1) is a flavonoid that isolated from leaves of *persimmon* or *Rosa agrestis*, and it was widely distributed in tea and has been used for treating many diseases for a long time as a traditional Chinese medicine [15,16]. Several studies have been confirmed that astragalin exhibited a number of biological properties, including anti-inflammatory, antioxidant, and antiatopic dermatitis effects [16–19]. It has been reported that astragalin exerted anti-inflammatory effects and showed to suppress the production of inflammatory cytokines in mouse peritoneal macrophages and acute lung injury through downregulating NF-κB signaling pathway [16,17]. Nevertheless, as expounded previously, the effect of astragalin on inflammatory response in LPS-induced mammary epithelial cells still need to be further understood. Thus, in this study we used primary cultured mouse mammary epithelial cells (mMECs) to further investigate the anti-inflammatory effect of astragalin and the underlying mechanisms.

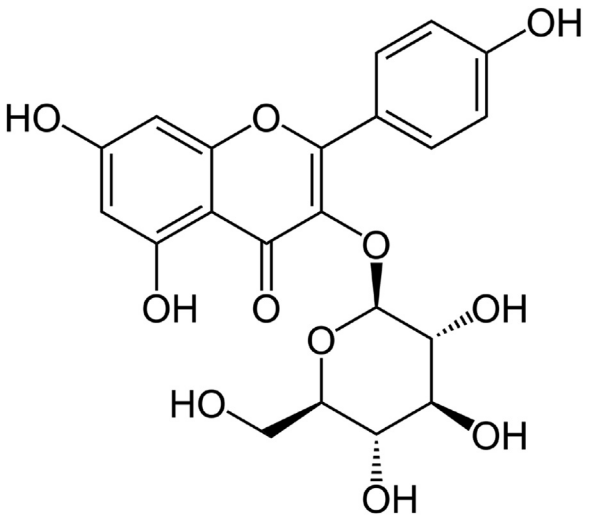

Fig. 1 – The chemical structure of astragalin.

2. Materials and methods

2.1. Chemicals and reagents

Astragalin (purity >99.9%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) (Fig. 1) and dissolved in dimethyl sulfoxide (Sigma) before use. The dimethyl sulfoxide concentration in the working solutions was <0.1%, which had no effect on present study. LPS (*E coli*, 055:B5) and phenylmethyl sulfonylfluoride was purchased from Sigma Chemical Co (St. Louis, MO). Cell Counting Kit-8 (CCK-8) was provided by Dojindo Laboratories (Kumamoto, Japan). Dulbecco's modified Eagle's medium/nutrient mixture F12 Ham (DMEM: F12/1:1), fetal bovine serum (FBS) and trypsin/ethylenediaminetetraacetic acid were obtained from HyClone (Logan, UT). Collagenases I and II were provided by Invitrogen (Carlsbad, CA). Epidermal growth factor, transferrin, and T3 were purchased from PeproTech Inc (Rockville, MD). Mouse TNF-α, IL-6, and enzyme-linked immunosorbent assay (ELISA) kits were obtained from BioLegend (San Diego, CA). Rabbit mAb IκBα, mouse mAb p65, p38, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), phosphor-IκBα, phosphor-p65, phosphor-p38, phosphor-ERK,