Cytokine xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Cytokine

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

Luteolin attenuates acute lung injury in experimental mouse model of sepsis

Soya Rungsung[a], Thakur Uttam Singh[a,*], Dipankar Jyoti Rabha[a], Tarun Kumar[a], Madhu Cholenahalli Lingaraju[a], Subhashree Parida[a], Avishek Paul[b], Monalisa Sahoo[c], Dinesh Kumar[a]

[a] Division of Pharmacology and Toxicology, ICAR-Indian Veterinary Research Institute, Izatnagar-243122, Bareilly, Uttar Pradesh, India
[b] Division of Physiology and Climatology, ICAR-Indian Veterinary Research Institute, Izatnagar-243122, Bareilly, Uttar Pradesh, India
[c] Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar-243122, Bareilly, Uttar Pradesh, India

ARTICLE INFO

Keywords:
Lung injury
Sepsis
Mice
Luteolin

ABSTRACT

The present investigation was undertaken to assess the result of pretreatment of luteolin in sepsis-induced acute lung injury in mice and its mechanism of action. Luteolin was administered intraperitoneally one hour before caecal ligation and puncture (CLP) surgery in mice. Acute lung injury was assessed by estimation of different parameters like lung edema, protein content, cytokines level, oxidative stress, inducible nitric oxide synthase (iNOS), intercellular adhesion molecule (ICAM)-1 expression and histopathology. Pretreatment of mice with luteolin showed decrease lung edema and protein content in tissue and bronchoalveolar lavage fluid (BALF). However, mice pretreated with luteolin showed reduction ($p = 0.92$) in blood and lung tissue bacterial counts however it was non significant. Further, luteolin showed significant reduction in interleukin (IL)-6 and IL-1β in lung tissue which are the proinflammatory cytokines. However, plasma IL-1β and tissue tumor necrosis factor (TNF)-α level decrease ($p = 0.24$; $p = 0.19$) with this pretreatment. Further, ICAM-1 mRNA expression and nuclear factor (NF)-kappa B protein expression were significantly ($p < 0.01$) decreased in luteolin pretreated septic mice. The lung iNOS level, iNOS mRNA and protein expressions were markedly ($p = 0.25$; $p = 0.50$; $p = 0.06$) altered with luteolin pretreatment, respectively. Also, significant reduction in lipid peroxidation and increase in the activity of antioxidant enzymes like superoxide dismutase (SOD) and catalase was noted with luteolin pretreatment. However, luteolin did not alter ($p = 0.36$) the non enzymatic antioxidant GSH activity in septic mice. Histopathology of lung tissue showed reduction in lung injury with the luteolin pretreatment in septic mice. The study suggests that luteolin showed attenuation in sepsis-induced acute lung injury in mice through suppression in ICAM-1, NF-kappa B, oxidative stress and partially iNOS pathways.

1. Introduction

Sepsis is a complicated state of immuno-inflammatory dysfunction which is typically an outcome of unsuccessful containment of infection [1]. Sepsis and its associated multi-organ failure and injury are a enormous burden with significant morbidity and mortality [2,3]. Sepsis often progresses to acute lung injury (ALI) marked by hypoxemia, acute respiratory distress syndrome (ARDS), and bilateral pulmonary infiltration [4,5]. Further, severe lung inflammation, alveolar flooding with proteinaceous substances, enhanced vascular permeability, and reduced lung compliance are hallmark features of ALI in septic condition [6]. The widespread vascular endothelial injury is considered a major contributor to multiple organ dysfunction and acute respiratory distress in sepsis which is responsible for augmentation of permeability of alveolar capillaries with protein-rich substances into airspaces [7]. Highest incidence of ALI are reported to occur in patients suffering with sepsis [8] and associated with greater incidence of morbidity and mortality rates in intensive care units (ICUs) patients [9,10]. Oxidative stress is a causative agent in a number of pathological conditions, which includes airway inflammation that is responsible for the pathogenesis and aggravation of pulmonary disease. In critical conditions like sepsis or ALI, there is severe generation of various reactive oxygen species (ROS) culminating to oxidative stress. Despite various therapeutic strategies for ALI such as nitric oxide, surfactant, glucocorticoids but none of them reduce the mortality in the sepsis-induced ALI/ARDS [11,12].

Plant-derived foods containing flavonoids showed protection on human health since last many years. Luteolin is a well known and most common flavonoid found in many edible foods including green pepper, broccoli, olive oil, celery, parsley and dandelion [13]. Luteolin exhibits

* Corresponding author.
E-mail address: tusingh80@gmail.com (T.U. Singh).

https://doi.org/10.1016/j.cyto.2018.03.042
Received 27 October 2017; Received in revised form 24 March 2018; Accepted 30 March 2018

various pharmacological activities such as anti-inflammatory [13], cardioprotective [14], antidiabetic [15] and antioxidant [16] effects. A study in lipopolysaccharide (LPS) model of ALI suggested that luteolin blocked the AKT/nuclear factor (NF)-kappa B pathway consequently suppressing inflammatory mediator expression [5]. Another recent report in endotoxin-induced lung injury, luteolin attenuated the pulmonary inflammatory response via inhibition of mitogen-activated protein kinase (MAPK) and NF-kappa B pathways [17]. Further, protective effects of luteolin against LPS-induced ALI through inhibition of mitogen activated protein kinase MEK/extracellular signal-regulated kinase (ERK) and phosphatidylinositol-3 kinase (PI3K)/Akt pathways have also been reported [18]. However, there is no report available on the sepsis-induced ALI in relation to luteolin pretreatment as the caecal ligation and puncture (CLP) model is the best natural model of sepsis. Therefore, present study was outlined with the objective to evaluate the preventive effects of luteolin against CLP-induced ALI in mice. Further, second objective was to explore the mechanism of action of preventive effect of luteolin, if any.

2. Material and methods

2.1. Experimental animals

Apparently healthy adult Swiss Albino male mice (25–30 g) procured from the Laboratory Animal Resource (LAR) Section, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, India were kept in polypropylene cages with free access to feed and water in Divisional animal house. All the experimental protocols and procedures were approved by Institute Animal Ethics Committee (IAEC).

2.2. Induction of CLP

Induction of sepsis was done by CLP technique in mice described by Wichterman and co-workers (1980) [19]. Animals were fasted overnight before the CLP surgery but with *ad libitum* access to water. Injections of xylazine (10 mg/kg body weight, intraperitoneally) and ketamine (100 mg/kg body weight, intraperitoneally) were administered for the induction of suitable anaesthesia. After induction of anesthesia, a ventral midline incision of about 2 cm was made and caecum was exposed and ligated with 3–0 silk distal to the ileocecal valve. The caecum was then punctured twice with 21-gauge needle, and returned to the abdomen closing the abdominal and skin incision in layers. To prevent dehydration post surgery, isotonic saline solution (1 ml/mouse) was administered subcutaneously to all mice. Sham-operated (sham) mice underwent the same surgical procedure except CLP surgery. All the surgical procedures were carried out as per the guidelines of IAEC.

2.3. Treatment

Animals were divided into four different groups: Group-I, Sham operated (sham); Group-II, Luteolin + Sham (Lut + sham); Group- III, sepsis (CLP); Group-IV, Luteolin + sepsis (Lut + CLP). Luteolin was administered intraperitoneally one hour before CLP surgery at the dose of 0.2 mg/kg body weight in Group-IV based on previous study [20]. Luteolin (0.2 mg/kg BW) was also provided to the Sham animal (Group-II).

2.4. Estimation of edema in lung tissue

An index of tissue water content/edema wet to dry weight ratio of the lungs was estimated. After sacrificing the animals, lung was excised, blot dried and further, it had been placed in pre-weighed glass plates. Wet weight of lung tissue was noted immediately. Lung tissues were then placed in a hot air oven at 70 °C for 48 h or until a stable dry weight. Lung tissue dry weight was then noted and expressed as % of Wet/Dry weight ratio (n = 6–7).

2.5. Estimation of protein content in lung tissue and bronchoalveolar lavage fluid (BALF)

20 ± 2 h post surgery, animals were anesthetized with injection of xylazine-ketamine i.p. and midline sternotomy was performed. Lungs were exposed and a small calibre tube was inserted into the airway. Thereafter, aliquots of 0.5 mM ethylenediaminetetraacetic acid (EDTA)-phosphate-buffered saline (PBS) about 0.5 ml were sequentially instilled, aspirated and pooled. The BALF samples were stored in ice for further analysis. Further, BALF (n = 7–13) and lung (n = 7–10) samples collected from different groups were centrifuged at 15,000 rpm for 10 min at 4 °C. Supernatant collected was stored in aliquots -20 °C till estimation of protein.

2.6. Pro-inflammatory cytokines estimation

20 ± 2 h post-surgery, blood samples (n = 5–6) and lung tissues (n = 4–11) from different groups of mice were collected. Plasma was separated by centrifuging at 1717*g* for 10 min and kept at -80 °C until further use. Tissue samples from lung were homogenized in PBS and centrifugated for 15 min at 1500*g* (or 5000 rpm). Supernatant was stored at −20 °C till assay. Interleukin (IL)-6 (Biospes, China), tumor necrosis factor (TNF)-α (Biospes, China) and IL-1β (Cusabio, USA) concentrations were estimated by enzyme-linked immunosorbent assay (ELISA) kits as per the manufacturer's instructions. Values were obtained from a standard curve and these values were expressed as pg/ml and pg/mg protein for plasma and tissue, respectively.

2.7. Determination of lung tissue iNOS level

Lung tissue samples (n = 5–8) were collected and rinsed with ice-cold PBS (0.02 mol/l, pH 7.0–7.2) to remove excess blood completely and weighed before homogenization in PBS. The homogenates were centrifugated for 15 min at 1500*g* (or 5000 rpm) and supernatant was stored at −20 °C. The iNOS level was estimated by ELISA (BlueGene, China) as per the manufacturer's instructions.

2.8. Oxidative stress and antioxidant enzymes

Malondialdehyde (MDA; n = 5–7) levels were estimated in tissue homogenate by thiobarbituric acid reactive substances (TBARS) method [21].Tissue reduced glutathione (GSH; n = 5–7) activity was assessed spectrophotometrically by 5 ,5'-dithiobisnitro benzoic acid (DTNB) method of Sedlak and Lindsay (1968) [22]. Superoxide dismutase (SOD; n = 5–6) was estimated as per the method described by Madesh and Balsubramanian (1998) [23]. Catalase (n = 4–5) was also assayed spectrophotometrically as described by Aebi (1983) [24].

2.9. Quantitative estimation of mRNA.

2.9.1. Isolation of mRNA

Lungs from different groups of animals (n = 3–4) were collected in 0.1% diethyl pyrocarbonate-treated autoclaved PBS at 20 ± 2 h post surgery and these tissues were stored in RNA later at −80 °C. Total RNA was isolated as per the manufacturer's instructions with Trizol (Ambion, Life Technologies, USA). The collected samples were treated with RNase free-DNase and the DNase was later inactivated by heating at 56 °C for a period of 10 min and chilled to 4 °C temperature. With the help of NanoDrop® spectrophotometer (Thermo Scientific, USA), purity of the RNA was quantified and further, samples obtained as RNA with an A260/A280 ratio of 1.8–2.0 were used for reverse transcription process using Revertaid® First strand cDNA synthesis kit (Thermo Scientific, USA) as per the manufacturer's instructions for cDNA synthesis.