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PROTECTIVE EFFECT OF HONOKIOL AGAINST LPS-INDUCED LUNG INJURY VIA ATTENUATION OF MATRIX METALLOPROTEINASE-9 AND OXIDATIVE STRESS

Hong-Bo Li[1,2], Lin Wang[3], Zheng-Tao Gu[1], Xuan He[1] and Lei Su[4,*]

[1] *Southern Medical University*, Guangzhou 510010, China

[2] *Department of ICU, General Hospital of Guangzhou Military Command*, Guangzhou 510010, China

[3] *Department of Emergency, First Affiliated Hospital of Guangzhou University of Chinese Medicine*, Guangzhou 510010, China

[4] *Department of ICU, General Hospital of Guangzhou Military Command, Key Laboratory of Tropical Zone Trauma Care and Tissue Repair of PLA*, Guangzhou 510010, China

***Corresponding author:** suleill27@hotmail.com

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Abstract: Despite high morbidity and mortality, no effective options are available for the treatment of acute lung injury (ALI). Therefore, the present study investigated the protective effect of honokiol (HK) on ALI via determination of its effect on several key biomarkers. The results of the study showed that HK significantly inhibited the infiltration of neutrophils and protein leakage induced by lipopolysaccharide (LPS) ($p<0.05$). The pretreatment with HK considerably boosted the endogenous antioxidant defense system to counteract the oxidative stress in LPS-induced ALI by elevating the levels of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH). Moreover, the activity of toxic mediators, such as myeloperoxidase (MPO), and lipid peroxidation were significantly inhibited upon treatment with HK. In order to examine the mechanism of action of HK, its effect was quantified using matrix metalloproteinase-9 (MMP-9) activity in bronchoalveolar lavage fluid (BALF) by gelatin zymography. Pretreatment with HK considerably suppressed the activation of MMP-9 in a concentration-dependent manner. These findings suggest that HK protects from lung injury via inhibition of MMP-9, and by enhancing the activity of the endogenous antioxidant defense system.

Key words: acute lung injury; honokiol; inflammation; MMP-9; oxidative stress

INTRODUCTION

The phrase acute respiratory distress syndrome (ARDS) was first applied by Ashbaugh and colleagues in 1967 to a cohort of 12 critically ill patients with acute respiratory failure [1] and subsequently, acute lung injury (ALI) was referred to, considered as the principal cause of ARDS. ALI was defined by an event of clinical complications including tachypnoea, hypoxemia, diffuse alveolar infiltrates, decreased pulmonary compliance and noncardiogenic pulmonary edema, resulting in a high incidence of morbidity and mortality. In the USA alone, around 200 000 people a year are affected by ARDS [2]. For the past two decades, mechanical ventilation has served as a protective strategy in treating ARDS patients [3]. Regardless of a better understanding of the underlying mechanism of ARDS, none of the pharmacological treatments offer a survival benefit in patients affected by it. Thus, plant-based natural products offer a selective advantage in treating this condition through the inhibition of oxidative stress and various inflammatory mediators [4].

Honokiol (HK), a biphenolic lignan isolated from *Magnolia officinalis* bark is most widely used in traditional Chinese medicine for "treating symptoms due to stagnation of Qi," as well as stress-related symptoms [5]. It has shown a wide array of biological activity without appreciable toxicity. Various studies suggest that HK possesses powerful antiproliferative action by inhibition of angiogenesis, apoptosis promotion, suppression of various cancer cell-signaling pathways, control of gene expression, enhancing the pharmacological activity of chemotherapeutic drugs and radiosensitization of cancer cells to radiation therapy. HK also shows a prospective beneficial effect in improving the health of an individual by reducing inflammation and oxidative stress. In mental illness, HK provides neurological protection, it is capable of managing

the glucose levels, and as a result, it helps to mitigate anxiety and depression [6-21]. However, no study has reported the effect of HK on ALI. Therefore, the aim of the present study was to determine the effect of HK on LPS-induced ALI in mice.

MATERIALS AND METHODS

Materials

Lipopolysaccharide (LPS; *Escherichia coli*, serotype 0111:B4), dimethyl sulfoxide (DMSO) and other reagents were procured from Sigma-Aldrich (USA). The concentration of DMSO was below 0.5% in the reaction mixture in all experiments. Kits used to assess the levels of CAT, SOD and GSH activities were obtained from Cayman (USA). The malondialdehyde (MDA) assay kit was obtained from ZeptoMetrix (USA).

Animals

Male mice weighing 25-30 g were used in the study. Animals were kept under a standard 12:12 hour light:dark cycle at 25°C and 45-50% relative humidity, and fed with standard laboratory chow and water *ad libitum*. The study was approved by the institutional Ethical Committee.

Murine model of LPS-induced lung injury

Forty mice were indiscriminately divided into 5 groups, including a control and four treatment groups. The control group received the vehicle intraperitoneally (i.p.) for 30 min, followed by intratracheal (i.t.) instillation of 50 µl saline. The mice in the four treatment groups received LPS (100 µg/50 µl), and were injected with 0, 10 and 50 µg/kg of HK or 1 mg/kg of dexamethasone (DEX; i.p.). After 6 h, the mice were anesthetized with sodium pentobarbital, killed, and samples were collected. In each group, the left lung of 4 animals was excised and collected for CAT, SOD and GSH activity assays.

Bronchoalveolar lavage fluid collection

After euthanasia, the trachea of the mice was uncovered and intubated with a tracheal cannula. BALF collection was performed by cleaning the airways and lungs with 1 mL of cold saline three times. The resultant liquid was pooled and kept on ice and then centrifuged at 500×*g* for 5 min at 4°C. Additionally, the cell-free supernatant was kept at -20°C for determination of the concentration of protein and estimation of MMP activity. Bio-Rad protein assay reagents were used for the estimation of protein concentrations in the cell-free BALF. Giemsa stain was used to calculate the total leukocyte content.

Assay of lipid peroxidation and antioxidant enzymes

The thiobarbituric acid reactive substances (TBARS) assay kit was used according to the standard protocol as per the manufacturer's instructions to quantify the lipid peroxidation products (MDA equivalents). CAT, SOD and GSH activities were determined using commercially available assay kits.

Determination of myeloperoxidase (MPO) activity

For MPO estimation, the lungs of the experimental rats were homogenized in 0.1 M phosphate buffer (pH 7.0) containing 0.5% cetyltrimethylammonium bromide. In brief, the supernatant was mixed with 1.5 mL phosphate buffer containing 0.2% cetyltrimethylammonium bromide in the presence of 10 mM guaiacol. The reaction was triggered by the addition of 0.01% H_2O_2. The absorbance of the sample was recorded using a microplate reader and translated as the specific activity of MPO in the tissue (U/mg).

Effect of HK on matrix metalloproteinase-9 activity

The gelatin zymography protease assay was used for the estimation of MMP-9 activity in the BALF. The BALF was solubilized in sodium dodecyl sulfate (SDS) sample buffer (63 mM Tris-HCl at pH 6.8, 10% glycerol, 2% SDS and 0.0025% bromophenol blue; after the addition of gelatin to 0.1%, the sample was subjected to 8% SDS polyacrylamide gel electrophoresis (PAGE). After electrophoresis, the gels were washed twice in 2.5% Triton X-100 for 1 h and incubated at 37°C for 16 h in reaction buffer (40 mM Tris-HCl pH 8.0, 10

mM $CaCl_2$, and 0.01% NaN_3). The gels were stained with Coomassie Brilliant R-250 and destained in a solution containing 7.5% acetic acid and 5% methanol.

Statistical analysis

Statistical analyses were performed using ANOVA followed by the Bonferroni *t*-test for multigroup comparisons; $p<0.05$ was considered significant for all tests. Data are expressed as means±standard deviation. The statistical analysis was performed with the help of GraphPad Prism Software 5.

RESULTS AND DISCUSSION

Effect of HK on LPS-induced ALI in mice

The LPS-induced ALI model mimics the clinical features of ALI found in humans but does not entirely replicate the characteristics of ALI. In mice, after instillation of LPS, the permeability of the alveolar-capillary barrier in lung tissues is increased and leads to infiltrations of leukocytes and leakage of protein [22]. Thus, we evaluated the effect of HK on the above-mentioned parameters by quantifying the total protein concentration in cells. As can be seen in Fig. 1, the protein concentration was significantly elevated in the BALF obtained from LPS-treated groups in comparison to the control ($p>0.05$). After pretreatment with HK, the level of LPS-induced injury was lowered. Regarding the effect of HK on infiltrations of leukocytes, it was found that HK prominently reduced the concentration of LPS-induced leukocyte infiltration in a dose-dependent manner. The results of the study suggest that HK showed a protective effect in ALI in mice.

Effect of HK on LPS-induced oxidative stress in lungs

Oxidative stress generated by the activation of neutrophils plays a vital role in injury of tissue [23]. Therefore, to prevent this, tissues have their own antioxidant defense mechanism that leads to the generation of endogenous chemicals such as SOD, CAT and GSH during healing from ALI. It was found that in the LPS-treated groups, the level of these enzymes were

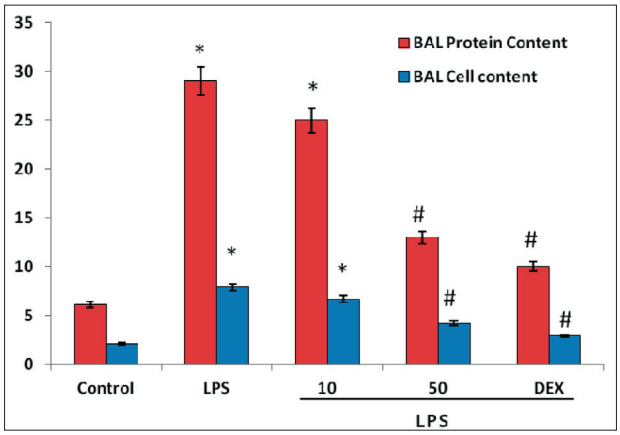

Fig. 1. Effect of HK on LPS-induced accumulation of protein and infiltration of leukocytes in BALF. Pulmonary infiltration was assessed by determination of the protein content in cell-free BALF (mg/mL) and the leukocyte count by determination of the cell content (10^6 cell/mL) in the BALF. The results are expressed as means±SD. *Variations among the specified and normal control and between the #LPS-treated groups, $p<0.05$.

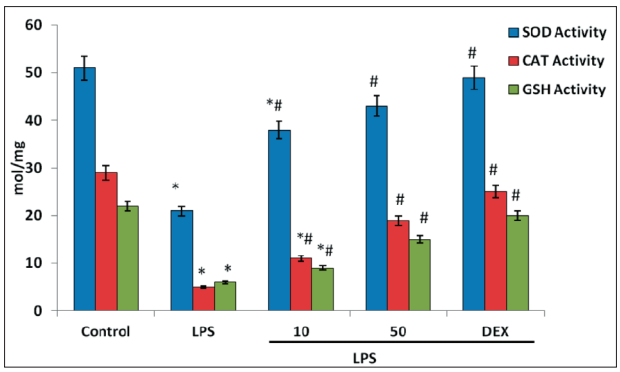

Fig. 2. The effect of HK on antioxidative enzymes (SOD, CAT, GSH) in lung tissue. The values are expressed as means±SD. *Difference between the specified and standard control group and between the #LPS-treated groups, $p<0.05$.

depressed, whereas as a result of drug activity, the levels were considerably increased (Fig. 2). It was noted that as the concentration rose, the activity also rose. Thus, it could be inferred that the administration of HK considerably reduced the LPS-induced oxidative stress.

Effects of HK on LPS-induced MPO activity in BALF

MPO is a toxic mediator and biomarker released during the degranulation process of neutrophils under the influence of infection [24]. MPO is mostly found in primary matrix granules responsible for the genera-

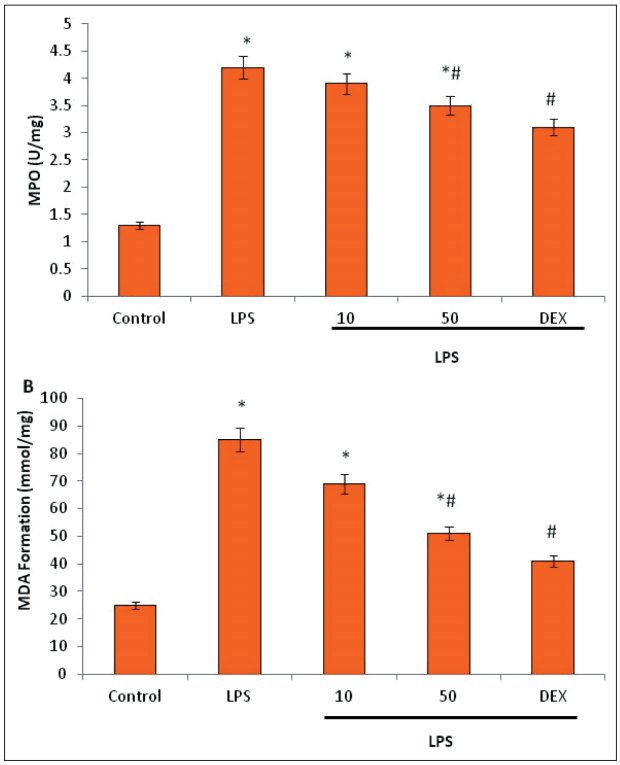

Fig. 3. The effect of HK on MPO activation and MDA formation. The data are presented as means±SD. *Difference between the specified and standard control group and between the #LPS-treated groups, p<0.05.

tion of various tissue-injury mediators. Thus, regulation of MPO could have protective effects on injury through the prevention of the generation of toxic mediators. As shown in Fig. 3A, in the LPS-treated groups the activity of MPO was found to be significantly higher than in the control (p<0.05). On the other hand, when pretreated with different concentrations of HK, the activity of MPO was reduced considerably. Thus, it can be surmised that HK plays a protective role against LPS-induced neutrophil activation.

Effect of HK on lipid peroxidation in LPS-induced ALI in mice

The intensity of oxidative stress was assessed by examining the change in the level of lipid peroxidation [25]. As shown in Fig. 3B, the level of lipid peroxide was found to be significantly elevated in the LPS-treated groups compared to the control (p<0.05). The level of peroxidation was considerably reduced with the introduction of HK, suggesting it has a role in the reduction of LPS-mediated injury.

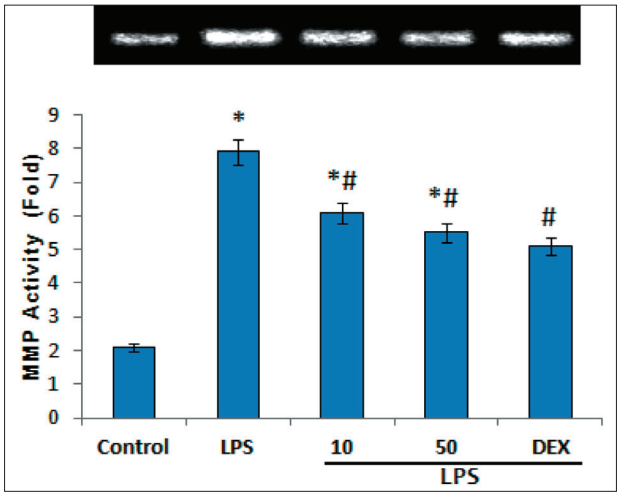

Fig. 4. The effect of HK on LPS-induced MMP-9 activation examined by zymography. The results of zymography (upper panel) were quantified and expressed as means±S.D. *Difference between the specified and standard control group and between the #LPS-treated groups, p<0.05.

Effects of HK on MMP-9 activity in the BALF of LPS-induced ALI mice

In an LPS-stimulated ALI model, MMP-9 or gelatinase B plays a vital role in the progression of lung injury via the regulation of NF-κB [26], which is produced after infection by activated neutrophils and found at elevated concentrations in the BALF of LPS-treated mice [27]. Thus, using gelatin zymography, the effect of HK was quantified by examining MMP-9 activity in the BALF (Fig. 4). It was found that the activity of MMP-9 was significantly elevated in the LPS-treated groups, in contrast to the control group (p<0.05). However, pretreatment with HK considerably suppressed the activation of MMP-9 in a concentration-dependent manner.

The presented findings show that HK exerts a protective effect in LPS-induced ALI through the inhibition of pathological pathways and by boosting the endogenous antioxidant defense system to counteract the oxidative stress in LPS-induced ALI.

Authors' contributions: H-B. Li and L. Wang performed the LPS-induced lung injury, Z-T. Gu performed the lipid peroxidation assay, X. He examined MPO activity and L. Su performed the statistical analysis. The final version of the article was approved by all the authors.

Conflicts of interest disclosure: The authors declare no conflict of interest.

REFERENCES

1. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. Lancet. 1967;2:319-23.
2. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ, Hudson LD, Incidence and outcomes of acute lung injury. N Engl J Med. 2005;353:1685-93.
3. Kress JP, Pohlman AS, O'Connor MF, Hall JB. Daily interruption of sedative infusions in critically ill patients undergoing mechanical ventilation. N Engl J Med. 2000;342:1471-7.
4. Brewer MS. Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. Compr Rev Food Sci F. 2011;10:221-47.
5. Fujita M, Itokawa H, Sashida Y. [Studies on the components of Magnolia obovata Thunb, 3: occurrence of magnolol and honokiol in M. obovata and other allied plants]. Yakugaku Zasshi. 1973;93:429-34.
6. Lin JW, Chen JT, Hong CY, Lin YL, Wang KT, Yao CJ, Lai GM, Chen RM. Honokiol traverses the blood-brain barrier and induces apoptosis of neuroblastoma cells via an intrinsic bax-mitochondrion-cytochrome c-caspase protease pathway. Neuro Oncol. 2012;14:302-14.
7. Xu D, Lu Q, Hu X. Down-regulation of P-glycoprotein expression in MDR breast cancer cell MCF-7/ADR by honokiol. Cancer Lett. 2006;243:274-80.
8. Wang X, Duan X, Yang G, Zhang X, Deng L, Zheng H, Deng C, Wen J, Wang N, Peng C, Zhao X, Wei Y, Chen L. Honokiol crosses BBB and BCSFB, and inhibits brain tumor growth in rat 9L intracerebral gliosarcoma model and human U251 xenograft glioma model. PLoS One. 2011;6:e18490.
9. Xu HL, Tang W, Du GH, Kokudo N. Targeting apoptosis pathways in cancer with magnolol and honokiol, bioactive constituents of the bark of Magnolia officinalis. Drug Discov Ther. 2011;5:202-10.
10. Arora S, Bhardwaj A, Srivastava SK, Singh S, McClellan S, Wang B, Singh AP. Honokiol arrests cell cycle, induces apoptosis, and potentiates the cytotoxic effect of gemcitabine in human pancreatic cancer cells. PLoS One. 2011;6:e21573.
11. Singh T, Prasad R, Katiyar SK. Inhibition of class 1 histone deacetylases in non-small cell lung cancer by honokiol leads to suppression of cancer cell growth and induction of cell death in vitro and in vivo. Epigenetics. 2013;8:54-65.
12. Ishikawa C, Arbiser JL, Mori N. Honokiol induces cell cycle arrest and apoptosis via inhibition of survival signals in adult T-cell leukemia. Biochim Biophys Acta. 2012;1820:879-87.
13. Kaushik G, Ramalingam S, Subramanium D, Rangarajan P, Protti P, Rammamoorthy P, Anant S, Mammen JM. Honokiol induces cytotoxic and cytostatic effects in malignant melanoma cancer cells. Am J Surg. 2012;204:868-73.
14. Hahm ER, Singh SV. Honokiol causes G0-G1 phase cell cycle arrest in human prostate cancer cells in association with suppression of retinoblastoma protein level/phosphorylation and inhibition of E2F1 transcriptional activity. Mol Cancer Ther. 2007;6:2686-95.
15. Ahn KS, Sethi G, Shishodia S, Sung B, Arbiser JL, Aggarwal BB. Honokiol potentiates apoptosis, suppresses osteoclastogenesis, and inhibits invasion through modulation of nuclear factor-kappaB activation pathway. Mol Cancer Res. 2006;4:621-33.
16. Wen J, Fu AF, Chen LJ, Xie XJ, Yang GL, Chen XC, Wang YS, Li J, Chen P, Tang MH, Shao XM, Lu Y, Zhao X, Wei YQ. Liposomal honokiol inhibits VEGF-D-induced lymphangiogenesis and metastasis in xenograft tumor model. Int J Cancer. 2009;124:2709-18.
17. Liu SH, Wang KB, Lan KH, Lee WJ, Pan HC, Wu SM, Peng YC, Chen YC, Shen CC, Cheng HC, Liao KK, Sheu ML. Calpain/SHP-1 interaction by honokiol dampening peritoneal dissemination of gastric cancer in nu/nu mice. PLoS One. 2012;7:e43711.
18. Steinmann P, Walters DK, Arlt MJ, Banke IJ, Ziegler U, Langsam B, Arbiser J, Muff R, Born W, Fuchs B. Antimetastatic activity of honokiol in osteosarcoma. Cancer. 2012;118:2117-27.
19. Fried LE, Arbiser JL. Honokiol, a multifunctional antiangiogenic and antitumor agent. Antioxid Redox Signal. 2009;11:1139-48.
20. Read AP, Strachan T. Cancer genetics. In: Human Molecular Genetics 2. New York, NY: Wiley; 1999. 427-42.
21. Cho JH, Jeon YJ, Park SM, Shin JC, Lee TH, Jung S, Park H, Ryu J, Chen H, Dong Z, Shim JH, Chae JI. Multifunctional effects of honokiol as an anti-inflammatory and anti-cancer drug in human oral squamous cancer cells and xenograft. Biomaterials. 2015;53:274-84.
22. Grommes J, Soehnlein O. Contribution of neutrophils to acute lung injury. Mol Med. 2011;17:293-307.
23. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal. 2014;20:1126-67.
24. Kuo MY, Liao MF, Chen FL, Li YC, Yang ML, Lin RH, Kuan YH. Luteolin attenuates the pulmonary inflammatory response involves abilities of antioxidation and inhibition of MAPK and NF-κB pathways in mice with endotoxin-induced acute lung injury. Food Chem Toxicol. 2011;49:2660-6.
25. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med. 1990;9:515-40.
26. Corbel M, Boichot E, Lagente V. Role of gelatinases MMP-2 and MMP-9 in tissue remodeling following acute lung injury. Braz J Med Biol Res. 2000;33:749-54.
27. Kang JL, Lee HW, Lee HS, Pack IS, Chong Y, Castranova V, Koh Y. Genistein prevents nuclear factor-kappa B activation and acute lung injury induced by lipopolysaccharide. Am J Respir Crit Care Med. 2001;164:2206-12.