科技部補助專題研究計畫成果報告
期末報告

研究刺槐素的急性肺損傷保護機制

計 畫 類 別 ： 個別型計畫
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執 行 單 位 ： 中山醫學大學醫學系藥理學科

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報 告 附 件 ： 出席國際學術會議心得報告

中 華 民 國 105 年 10 月 25 日

中 文 摘 要 ： 背景：急性肺損傷為常見且嚴重的肺部發炎性疾病，具有高的發生率與死亡率。且至今為止，臨床上仍無有效治療急性肺損傷的對策。刺槐素為一類黃酮類化合物，主要存在於各式植物之中，包括有雪蓮、大薊、苦林盤、菊科植物之中。過去文獻已證實刺槐素有效降低多種發炎相關性的疾病，如發炎性疼痛、巴金森氏症、氣喘、與癌症。本研究計畫利用一年度的時間，進行刺槐素對於脂多醣引發的急性肺損傷疾病與其內的分子機制。
材料與方法：本研究主要利用脂多醣直接肺腔注射進入肺部，引發的急性肺損傷動物模式進行研究。並於脂多醣注射前 30 分鐘，投入不同濃度的刺槐素 (0, 1, 10, 100 mol/kg) 以腹腔注射投予。當脂多醣投予 6 小時後，犧牲動物後採取各式檢體。將收集肺泡沖出液，分析促發炎激素表現量、粘附分子表現量與白血球計數與分型。肺組織的檢體部分，進行肺組織病理學分析、脂質過氧化分析、抗氧化酵素與蛋白質活性分析、與其他調節分子磷酸化分析。
結果：刺槐素依濃度依存性的方式降低了，由脂多醣引發的肺部病理組織的變化、白血球浸潤。再者，刺槐素降低了促發性細胞素與粘降分子的表現與肺臟脂質的過氧化作用。然而、刺槐素依濃度依存性的方式提高由脂多醣所引發的抗氧化酵素降低的反應，包括有超氧分歧酶、觸酶、穀胱甘肽過氧化物酶。脂多醣所引發 p38 MAPK, ERK, JNK 磷酸化反應也可受到刺槐素的抑制。最後，刺槐素可抑制 NF-κB 磷酸化與 IκB 降解反應。
結論：刺槐素可有效降低由脂多醣所引發的急性肺損傷作用。其中主要的分子機制為提高抗氧化酵素的活性與降低 MAPK- NFκB 分子機制。

中 文 關 鍵 詞 ： 刺槐素; 急性肺損傷; 促發炎介質; 抗氧化酵素; MAPK; NFκB

英 文 摘 要 ： Abstract
Background: Acute lung injury (ALI), the serious and acute pulmonary inflammatory disorder, remains the high incidence and mortality in patients. Up to now, there are no effective therapy strategies available clinically for the improvement of ALI. Acacetin, belonging to the family of flavonoids, is present in a vast of plants, such as Saussurea involucrata Cirsium rhinoceros, Clerodendrum inerme (L.) Gaertn, Compositae. Acacetin has been shown to cause beneficial effects against inflammation-related diseases such as inflammatory pain, Parkinson ‘s disease, asthma, and cancer. The aim of this study to investigate the potential protective effects of acacetin and the molecular mechanisms involved in lipopolysaccharide (LPS)-induced ALI.
Materials and Methods: In the mice ICR model, ALI was induced by intratracheal administration of LPS, and acacetin at various concentrations (0, 1, 10, 100 mol/kg) was intraperitoneal administration for 30 min prior to LPS treatment for 6 h. Brachoalveolar lavage fluid (BALF) was collection for leukocyte counts, proinflammatory cytokines

expression, and adhesion molecules expression. Lung tissues were isolated for histopathological analysis, measurement of lipid peroxidation and antioxidative enzymes activities, western blot analysis of regulatory molecular mechanism. Results: Pretreatment with acacetin inhibited histopathological changes and leukocytes infiltration in lungs in LPS-induced ALI in a concentration-dependent manner. Pretreatment with acacetin reduced the expression of proinflammatory cytokines and adhesion molecules in LPS-induced ALI in a concentration-dependent manner. LPS-induced lipid peroxidation is reduced by acacetin in a concentration-dependent manner. We found that decreased activities of superoxide dismutase, catalase, and glutathione peroxidase induced by LPS were reversed by acacetin in a dose-dependent manner. Phosphorylation of p38 MAPK, ERK and JNK were inhibited by acacetin in LPS-induced ALI in a concentration-dependent manner. Phosphorylation of NF-κB and degradation of IκB were inhibited by acacetin in LPS-induced ALI in a concentration-dependent manner. Conclusion: These results from present study suggested that the protective mechanisms of acacetin on LPS-induced ALI were via up-regulation of antioxidative enzymes and down-regulation of MAPK-NFκB pathway.

英文關鍵詞：Acacetin; acute lung injury; proinflammatory mediators; AOE; MAPK; NFκB

科技部補助專題研究計畫成果報告

（□期中進度報告/■期末報告）

研究刺槐素的急性肺損傷保護機制

計畫類別：■個別型計畫　□整合型計畫
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計畫主持人：關宇翔
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計畫參與人員：李妙卿, 梁清惠

本計畫除繳交成果報告外，另含下列出國報告，共 _1__ 份：
□執行國際合作與移地研究心得報告
■出席國際學術會議心得報告

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中　華　民　國 105 年 10 月 19 日

中文摘要

背景：急性肺損傷為常見且嚴重的肺部發炎性疾病，具有高的發生率與死亡率。且至今為止，臨床上仍無有效治療急性肺損傷的對策。刺槐素為一類黃酮類化合物，主要存在於各式植物之中，包括有雪蓮、大薊、苦林盤、菊科植物之中。過去文獻已證實刺槐素有效降低多種發炎相關性的疾病，如發炎性疼痛、巴金森氏症、氣喘、與癌症。本研究計畫利用一年度的時間，進行刺槐素對於脂多醣引發的急性肺損傷疾病與其內的分子機制。

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結果：刺槐素依濃度依存性的方式降低了，由脂多醣引發的肺部病理組織的變化、白血球浸潤。再者，刺槐素降低了促發性細胞素與粘降分子的表現與肺臟脂質的過氧化作用。然而、刺槐素依濃度依存性的方式提高由脂多醣所引發的抗氧化酵素降低的反應，包括有超氧分歧酶、觸酶、穀胱甘肽過氧化物酶。脂多醣所引發 p38 MAPK, ERK, JNK 磷酸化反應也可受到刺槐素的抑制。最後，刺槐素可抑制 NF-κB 磷酸化與 IκB 降解反應。

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關鍵字
刺槐素；急性肺損傷；促發炎介質；抗氧化酵素；MAPK；NFκB

Abstract

Background: Acute lung injury (ALI), the serious and acute pulmonary inflammatory disorder, remains the high incidence and mortality in patients. Up to now, there are no effective therapy strategies available clinically for the improvement of ALI. Acacetin, belonging to the family of flavonoids, is present in a vast of plants, such as *Saussurea involucrata Cirsium rhinoceros, Clerodendrum inerme (L.) Gaertn*, Compositae. Acacetin has been shown to cause beneficial effects against inflammation-related diseases such as inflammatory pain, Parkinson's disease, asthma, and cancer. The aim of this study to investigate the potential protective effects of acacetin and the molecular mechanisms involved in lipopolysaccharide (LPS)-induced ALI.

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Results: Pretreatment with acacetin inhibited histopathological changes and leukocytes infiltration in lungs in LPS-induced ALI in a concentration-dependent manner. Pretreatment with acacetin reduced the expression of proinflammatory cytokines and adhesion molecules in LPS-induced ALI in a concentration-dependent manner. LPS-induced lipid peroxidation is reduced by acacetin in a concentration-dependent manner. We found that decreased activities of superoxide dismutase, catalase, and glutathione peroxidase induced by LPS were reversed by acacetin in a dose-dependent manner. Phosphorylation of p38 MAPK, ERK and JNK were inhibited by acacetin in LPS-induced ALI in a concentration-dependent manner. Phosphorylation of NF-κB and degradation of IκB were inhibited by acacetin in LPS-induced ALI in a concentration-dependent manner.

Conclusion: These results from present study suggested that the protective mechanisms of acacetin on LPS-induced ALI were via up-regulation of antioxidative enzymes and down-regulation of MAPK-NFκB pathway.

Keywords

Acacetin; acute lung injury; proinflammatory mediators; AOE; MAPK; NFκB

1. Introduction

Acute lung injury (ALI) and its severe form, acute respiratory distress syndrome, are two serious illnesses associated with acute pulmonary inflammatory responses which lead to respiratory failure. A recent study performed in the King County of Washington estimates the crude incidence of ALI to be 78.9 per 100,000 person-years, with an age-adjusted incidence of 86.2 per 100,000 person-years. Mortality rate for patients with ALI is about 40-60% and remains high although many new therapeutic strategies have been developed. The development of ALI is triggered by several important environmental risk factors which can be divided into direct and indirect factors (Johnson and Matthay, 2010). Sepsis and pneumonia are the common factors for causing indirect and direct lung injury respectively. Lipopolysaccharide (LPS) is a compound found in the outer cell membrane of gram-negative bacteria. LPS has been recognized as playing the pivotal role in the pathogenesis of sepsis and pneumonia. Therefore, LPS is a potent agent which induces inflammatory responses and plays as an important pathological factor in ALI (Johnson and Matthay, 2010).

After administration of LPS into the lungs, histopathological changes characterized by neutrophilic alveolitis, hyaline membrane formation, and increased alveolar barrier thickness occured (Grommes and Soehnlein, 2011). In the progression of ALI, neutrophils activation is the critical component of the innate immune system. Neutrophils engulf and kill the bacteria and fungi via phagocytosis, degranulation, and respiratory burst. However, oxidative stresses are generated from degranulation and respiratory burst and result in neighborhood tissue damages that lead to inflammation in lung (Grommes and Soehnlein, 2011).Among the proinflammatory mediators, including tumor necrosis factor (TNF)-α, interlukine (IL)-6, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1, from activation of alveolar macrophages and epithelial cells alter the integrity of alveolar-capillary barrier and activation of peripheral neutrophils (Beck-Schimmer et al., 2005). Nuclear factor (NF)-κB, a proinflammatory transcription factor, is one of the most important factors which participate in the regulation of proinflammatory mediators generation. Mitogen-activated protein kinases (MAPK), which including p38 MAPK, ERK and JNK have been demonstrated to modulate NF-κB activation in LPS induced ALI. The mechanism of protection against oxidative stress injury in lung tissue involves antioxidative enzymes (AOEs) including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and nuclear factor erythroid 2-related factor (Nrf2)/ heme oxygenase (HO-1) antioxidant pathway (Sun et al., 2016).

Up to now, the incidence and mortality rate for ALI are still high. In the United States, the incidence of ALI is 200,000 patients per years with a mortality rate of 40% (Johnson and Matthay, 2010). However, the methods of effective treatment are still deficiency and development. Acacetin, chemical name: 4'-methoxy-5,7-dihydroxyflavone, is a O-methylated flavon and the main component isolated from the *Saussurea involucrata Cirsium rhinoceros*, *Clerodendrum inerme (L.) Gaertn*, Compositae. I Acacetin has been shown to cause beneficial effects against inflammation-related diseases such as inflammatory pain, Parkinson's disease, asthma, and cancer. The aim of this study to investigate the potential protective effects of acacetin and the molecular mechanisms involved in lipopolysaccharide (LPS)-induced ALI. .

2. Materials and methods

2.1. Materials

Catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) activity assay kits were products of Cayman (Ann Arbor, MI, USA) and the malondialdehyde (MDA) assay kit was manufactured by ZeptoMetrix (Buffalo, NY, USA). Antibodies against IκB, phospo-p65, p65, phospo-p38 MAPK, p38 MAPK,

phospo-ERK, ERK, phospo-JNK, JNK, and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Secondary antibodies were obtained from Jackson Immuno Research Laboratories (Baltimore, MD, USA). Lipopolysaccharide (LPS; *Escherichia coli*, serotype 0111:B4), dimethyl sulfoxide (DMSO), and other reagents, unless specifically stated elsewhere, were purchased from Sigma-Aldrich (St. Louis, MO, USA). The final volume of DMSO in the reaction mixture was <0.5%.

2.2. Mice and experimental design

Male ICR mice (8-10 weeks old) weighing 25-30 g were purchased from the BioLASCO (Taipei, Taiwan). Mice were housed and maintained at a constant temperature (21 ± 1°C) and humidity under a 12 hrs/12 hrs light dark cycle. Mice fed with the commercial diet and water available *ad libitum*. All procedures performed on the animals were approved by the Institutional Animal Ethics Committee of Chung Shan Medical University.

The animals (n = 60) were were randomly divided into six groups, including a control and five treatment groups. The mice of the control group received vehicle intraperitoneally (IP) followed 30 min later by intratracheal (IT) administration of 50 μl of saline; the mice of the five treatment groups were injected with acacetin at concentration of 0, 1, 10, or 100 μmol/kg IP followed 30 min later by IT instillation of 100 μg/50 μl of LPS. After 6 hours, the mice were killed by pentobarbital (40 mg/kg) IP and tissue samples were collected. In addition, bronchoalveolar lavage fluid (BALF) was collection and pooled together from the lungs.

2.3. Histopathological analysis

After sacrifice, the lungs were excised by midsternal thoracotomy and fixed via tracheal cannula with 4% paraformaldehyde. After the lungs were dehydrated and embedded in paraffin at 60°C, a 3 μm histological sections were procured using a rotatory microtome and stained with hematoxylin-eosin. Histopathological features of acute lung injury, including Haemorrhage, infiltration of leukocytes, change in the thickness of alveolar wall, and formation of hyaline membrane, were evaluated using light microscopy.

2.4. Bronchoalveolar lavage and Cell Counting

After sacrifice, the lungs were lavaged with sterile saline and cell numbers were counted as described previously. Briefly, the lungs were lavaged with 1 ml sterile saline three times via tracheal cannula. After centrifugation, the BALF was collected at 800 *g* for 10 min at 4 ℃. The pellets were resuspended and stained with Geimsa soultion for the cells counting under the microscope. In addition, the supernatant was stored at -20 ℃ for measurement of cytokines and adhesion molecules expression.

2.5. Measurement of cytokines and adhesion molecules expression.

The expression of cytokines, which including TNF-α and IL-6, and adhesion molecules, which including ICAM-1 and VCAM-1, in BALF supernatant were measured by ELISA assay kits (R & D Systems, Minneapolis, MN). The concentrations were interpolated from the standard curves for recombinant TNF-α, IL-6, ICAM-1, and VCAM-1.

2.6. Western blot analysis of lung tissue

After sacrifice, the lungs were harvested and frozen in liquid nitrogen immediately. Proteins were

extracted from the lungs which homogenized in tissue protein extraction solution (T-PER; Pierce, Rockford, IL). And then, the samples were separated by SDS–PAGE and transferred to polyvinylidene difluoride membrane. The membranes were blocked with 5% nonfat dried milk for 1 h. After washed by PBS including 0.1% Tween-20 (PBST), then probed with antibodies including IκB, phospo-p65, p65, phospo-p38 MAPK, p38 MAPK, phospo-ERK, ERK, phospo-JNK, JNK, and β-actin. After washed, the horseradish peroxidase-labeled IgG was added for 1 h, and the blots were developed using enhanced chemiluminescence.

2.7. Measurement of lipid peroxidation

The levels of lipid peroxidation in lungs were measured by the thiobarbituric acid reactive substances (TBARS) assay as previously described. The results of TBARS were expressed as MDA formation, which was presented as mmol/mg of the protein.

2.8. Measurement of antioxidative enzymes activities

The activities of SOD, CAT, and GPx were determined using commercially available assay kits following the manufacturer's instructions and previous studies.

2.9. 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 were expressed as mean ± standard deviation (S.D.) of at least three separate experiments..

3. Results

3.1. Effects of acacetin on lung histopathology in LPS-induced ALI

To evaluate the effect of histopathological changes in the lungs after acacetin administration on LPS-induced ALI, the mice pretreated with acacetin at different concentrations before LPS administration. In the control group, as shown in Fig 1A, the sight histopathological alteration and normal structure were observation. In the LPS treated mice without acacetin pretreatment, as shown in Fig 1B, the lung specimens represented notably pathologic changes, including neutrophil infiltration, alveolar wall thickness, hemorrhage, and hyaline membrane formation. The LPS-induced pathologic changes were ameliorated by pretreatment with acacetin in the concentration-dependent manner, as shown in Fig 1C, D, and E. In contrast, LPS-induced pathologic changes also were reduced by dexamethasone, as shown in Fig 1F. The results suggested that acacetin has the ability to improvement the pathologic changes of the lungs in LPS-induced ALI murine model.

3.2. Effects of acacetin on leukocyte infiltration in LPS-induced ALI

Increased alveolar-capillary membrane permeability is one of important pathological characteristics and results in lung edema and leukocyte infiltration. Lung edema determined by the W/D ratio. Leukocyte infiltration was quantified by Giemsa stain. After LPS administration, extensive leukocyte infiltration into BALF was significantly increased, which compared to untreated control group ($p < 0.05$). Pretreatment with acacetin, LPS induced leukocytes infiltration was inhibited in a concentration-dependent manner, significant inhibitory effect began at 10 μmol/kg ($p < 0.05$) (Fig 2). These results indicated that the protective effect of acacetin in LPS-induced ALI mice.

3.3. Effects of acacetin on expression of adhesion molecules in LPS-induced ALI

Adhesion molecules, ICAM-1 and VCAM-1, participate in the recruitment of leukocytes in the lung in LPS-induced ALI. After LPS administration, the expression of ICAM-1 and VCAM-1 was significantly increased in BALF ($p < 0.05$). However, pretreatment with acacetin significantly inhibited the expression of ICAM-1 and VCAM-1 in a concentration-dependent manner with statistically significant inhibition from control at 10 μmol/kg ($p<0.05$) (Fig 3). These results indicated acacetin reduced leukocyte infiltration in LPS-induced ALI via down-regulation of adhesion molecules expression.

3.4. Effects of acacetin on expression of proinflammatory cytokines in LPS-induced ALI

Proinflammatory cytokines, TNF-α and IL-6, play an important role in LPS-induced ALI. After LPS administration, the expression of TNF-α and IL-6 was significantly increased in BALF ($p < 0.05$). However, pretreatment with acacetin significantly inhibited the expression of TNF-α and IL-6 in a concentration-dependent manner with statistically significant inhibition from control at 1 μmol/kg ($p<0.05$) (Fig 4). These results indicated acacetin reduced LPS-induced ALI via down-regulation of TNF-α and IL-6 expression.

3.5. Effects of acacetin on the NFκB phosphorylation and IκB degradation in LPS-induced ALI

NFκB has a critical role in expression of adhesion molecules and proinflammatory cytokines in LPS-induced ALI. After LPS administration, NFκB phosphorylation was significantly increased in the lungs when compared with control group ($p<0.05$). Pre-treatment with acacetin reduced the NFκB phosphorylation in a concentration-dependent manner starting at 10 μmol/kg ($p<0.05$, Fig. 5A). In parallel with NFκB phosphorylation, the effect of acacetin on IκB degradation was also investigated. Similar to NFκB phosphorylation, LPS induced degradation of IκB which was significantly inhibited by acacetin in a concentration-dependent manner with a significant inhibitory effect also starting from 10 μmol/kg ($p<0.05$) (Fig 5B). These results indicated that acacetin reduced LPS-induced expression of adhesion molecules and proinflammatory cytokines by reducing the NFκB phosphorylation and IκB degradation.

3.6. Effects of acacetin on the MAPK phosphorylation in LPS-induced ALI

The evidence has demonstrated that MAPK is the upstream factor in NFκB phosphorylation and IκB degradation in LPS-induced ALI. LPS stimulation significantly increased MAPK, including p38 MAPK, ERK, and JNK, phosphorylation in comparison to the control group ($p < 0.05$). Acacetin inhibited LPS-induced phosphorylation of MAPK in a concentration-dependent manner starting at 10 μmolg/kg ($p < 0.05$, Figure 6). These results indicated that acacetin inhibited LPS-induced NFκB phosphorylation by reducing the MAPK phosphorylation.

3.7. Effects of acacetin on LPS-decreased activation of antioxidative enzymes

Oxidative stress, which generated from neutrophils activation, plays an important role in the pathogenesis of LPS-induced ALI. Activation of antioxidative enzymes, including SOD, CAT, and GPx, are induced during recuperation from ALI. The activation of SOD, CAT, and GPx was significantly decreased in LPS-treated mice as compared to untreated group ($p < 0.05$). Pretreatment with acacetin restored LPS-induced reduction of the SOD, CAT, and GPx activities in a concentration-dependent manner, started at the

concentration of 10 μmol/kg ($p < 0.05$; Fig 7). These results suggested that acacetin was able to reduce LPS-induced oxidative stress.

3.8. Effects of acacetin on LPS-induced lipid peroxidation

MDA is the end product of lipid peroxidation, which is an important biomarker for damage by oxidative stress in tissue. The level of MDA generation in lungs was significantly higher in LPS treated mice as compared with untreated group ($p < 0.05$). Pretreatment with acacetin reduced LPS-induced MDA formation in a concentration-dependent manner. At both 10 and 100 μmol/kg, acacetin significantly attenuated lipid peroxidation in lung tissue ($p < 0.05$) (Fig. 8). These results indicated acacetin reduced LPS-induced lipid peroxidation.

4. Discussion

ALI is mostly casued by sepsis originating from gram-negative bacterial infection and is the cause of inceasing mortality and morbidity for sepsis. LPS is an ubiquitous and prominent component of the outer membrane in most gram-negative bacteria. There are two regions, lipid A and polysaccharide, in LPS. Lipid A is the primary target of the innate immune system, including macrophages and neutrophils (Raetz et al., 2007). After instillation of LPS into lung directly, several stages of inflammatory responses were induced in mice. At first, LPS activates the cells of the alveolocapillary barrier and alveolar macrophages via Toll-like receptors 4 and its cofactor, CD14. Secondly, these activated cells produce proinflammatory mediators via intracellular signal transduction. Then, proinflammatory cytokines induce the recruitment of peripheral leukocytes into the lung. In early time, neutrophils are the main type of leukocytes to act against the pathogen and break the alveolocapillary barrier via respiratory burst and degranulation. Finally, neutrophil infiltration and alveolocapillary barrier dysfunction lead to hypoxia, pulmonary edema, and hyaline membrane formation (Grommes and Soehnlein, 2011). The clinical and pathological features of ALI in humans are similar to LPS-induced ALI in the mouse model.[5] However, the development of an effective therapy for ALI is still in progress. Therefore, we carefully studied the effect of wogonin on the mouse model for ALI induced by intratracheal instillation of LPS in hope of establishing a potential compound against ALI. At present, we found that acacetin improved the LPS-induced histopathological changes, lung edema, and leukocytes infiltration. These results indicated that acacetin could be a potential preventive or therapeutic agent for ALI.

Neutrophil activation followed by migration into the alveolar space is an important step in the progression of LPS-induced ALI (Grommes and Soehnlein, 2011). In the histopathological experiment, we also found neutrophil infiltration in the LPS treated group which was reduced by zerumbone. Activation of neutrophils is associated with chemotaxis and degranulation. During chemotaxis and degranulation, the expression of adhesion molecules, including ICAM-1 and VCAM-1, are increased by LPS in ALI. In the present, we found that the expression of ICAM-1 and VCAM-1 was reduced in LPS-induced ALI in lungs with acacetin pretreatment when compared with the control group. These results indicated that acacetin improved the LPS-induced ALI by reducing expression of adhesion molecules.

At the inflammatory site, proinflammatory cytokines such as TNF-α and IL-6 are secreted from pulmonary cells and alveolar macrophages. These cytokines play an important role in LPS-induced ALI (Kuo et al., 2011). TNF-α is the early response cytokine generated by activated alveolar macrophages that appear in BALF and plasma in ALI. The secretion of TNF-α in turn stimulates the neighboring cells to generate more effective proinflammatory cytokines, such as IL-6 which subsequently mediate the recruitment of PMNs,

macrophages, and lymphocytes. In a murine model of LPS-induced ALI, acacetin reduced the production of TNF-α, IL-6 in BALF. The results indicate that acacetin reduced leukocyte infiltration into lung by decreasing the expression of proinflammatorycytokines and proteins.

The transcription factor NF-κB is the crucial signal factor modulating proinflammatory cytokines in LPS-induced ALI (Fan et al., 2001). NFκB is the transcription factor which serves as a primary regulator in inflammation, apoptosis, and proliferation. NFκB comprises five subunits, p65, Rel B, c-Rel, p50, and p52, which interact with each other to form active homo- or hetero-dimers. Among all forms of NFκB dimers, the heterodimer p65/p50 is the most abundant and ubiquitous in almost all cell types. Nuclear translocation of activated NFκB, which is phosphorylated on serine residues, is due to exposure to the nuclear location signal during IκB degradation (Oeckinghaus and Ghosh, 2009). In a murine model of LPS-induced ALI, the increase of NFκB phorphorylation and IκB degradation in LPS-treated groups is prevented by acacetin in a concentration-dependent manner. We suggest that the mechanism of the protective effect of acacetin on LPS-induced ALI was through the NFκB pathway. MAPK is pathways participate in the activation of NFκB in LPS-induced ALI (Li et al., 2012). In a murine model of LPS-induced ALI, the increase of MAPK phorphorylation in LPS-treated groups is prevented by acacetin in a concentration-dependent manner. We suggest that the mechanism of the inhibition of NFκB pathway of acacetin on LPS-induced ALI for the most part was through the MAPK phosphorylation. The results gathered from the present study suggest that acacetin could be an effective agent against endotoxin induced ALI via MAPK-NF NFκB pathway.

Antioxidants deplete and neutralize the ROS, thus reduce or delay the capacity of damages cause by ROS. There are several AOEs present in body, such as SOD, CAT, and GPx (Lobo et al., 2010). SOD converts the superoxide anions into hydrogen peroxidase and molecular oxygen. Then, CAT and GPx convert hydrogen peroxidase into water (Paiva and Bozza, 2014). At present, we found the activation levels of SOD, CAT, and GPx were reversed by acacetin in lung after LPS administration. In addition, we also found acacetin extract inhibited LPS-induced lipid peroxidation in lung of the mice ALI model. These results indicated that acacetin could be an effective agent against endotoxin induced ALI via upregulation the activation of antioxidative enzymes.

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Sun CY, Xu LQ, Zhang ZB, Chen CH, Huang YZ, Su ZQ, Guo HZ, Chen XY, Zhang X, Liu YH, Chen JN, Lai XP, Li YC, Su ZR. Protective effects of pogostone against LPS-induced acute lung injury in mice via regulation of Keap1-Nrf2/NF-κB signaling pathways. Int Immunopharmacol. 2016; 32: 55-61.

Figure legends

Figure 1 Effects of acacetin pretreatment on histopathological changes of lung tissues in LPS-induced ALI (100X). (A) Control (B) LPS (C) 1 μmol/kg acacetin + LPS (D) 10 μmol/kg acacetin + LPS (E) 100 μmol/kg acacetin + LPS.

Figure 2 Effects of acacetin on leukocyte infiltration in LPS-induced ALI. Leukocytes infiltration determined by leukocyte counts in BALF. Values are expressed as mean ± S.D. of 4 mice per group. # Represents significant difference between the indicated and normal control group; * between the indicated and LPS groups, $p < 0.05$.

Figure 3 Effects of acacetin on expression of adhesion molecules in LPS-induced ALI. Expression of adhesion molecules, including ICAM-1 and VCAM-1, in BALF was determined by ELISA assay. Values are expressed as mean ± S.D. of 4 mice per group. # Represents significant difference between the indicated and normal control group; * between the indicated and LPS groups, $p < 0.05$.

Figure 4 Effects of acacetin on expression of proinflammatory cytokines in LPS-induced ALI. Expression of proinflammatory cytokines, including TNF-α and IL-6, in BALF was determined by ELISA assay. Values are expressed as mean ± S.D. of 4 mice per group. # Represents significant difference between the indicated and normal control group; * between the indicated and LPS groups, $p < 0.05$.

Figure 5 Effect of acacetin on LPS-induced NFκB phosphorylation and IκB degradation in the lungs. After the lungs harvested from post-treated animals were analyzed by Western blotting. The fold changes of NFκB phosphorylation and IκB degradation between the treated and control groups were calculated. Values are expressed as mean ± S.D. of 4 mice per group. # Represents significant difference between the indicated and normal control group; * between the indicated and LPS groups, $p < 0.05$.

Figure 6 Effect of acacetin on LPS-induced MAPK phosphorylation in the lungs. After the lungs harvested from post-treated animals were analyzed by Western blotting. The fold changes of MAPK phosphorylation between the treated and control groups were calculated. Values are expressed as mean ± S.D. of 4 mice per group.. # Represents significant difference between the indicated and normal control group; * between the indicated and LPS groups, $p < 0.05$.

Figure 7 Effect of acacetin on LPS-induced antioxidative enzyme activation in lung tissue. The activated antioxidative enzymes included (A) SOD, (B) CAT, and (C) GPx. Values are expressed as mean ± SD of 3 mice per group. *Represents significant difference between the indicated and normal control group; #between the indicated and LPS groups, p <0.05.

Figure 8 Effects of zerumbone on LPS-induced MDA formation in lung tissue. Values are expressed as mean ± SD of 3 mice per group. *Represents significant difference between the indicated and normal control group; #between the indicated and LPS groups, p <0.05.

Fig 1

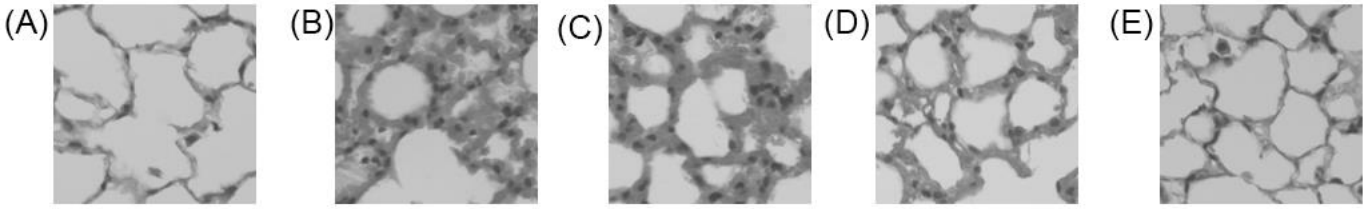


Fig 2

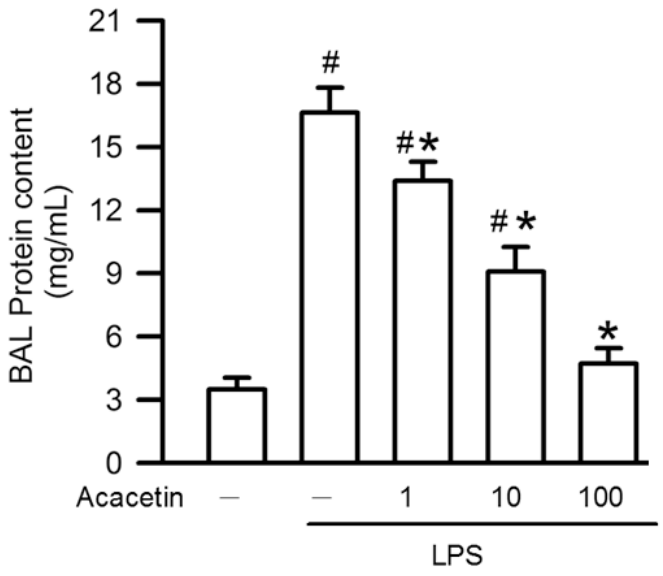
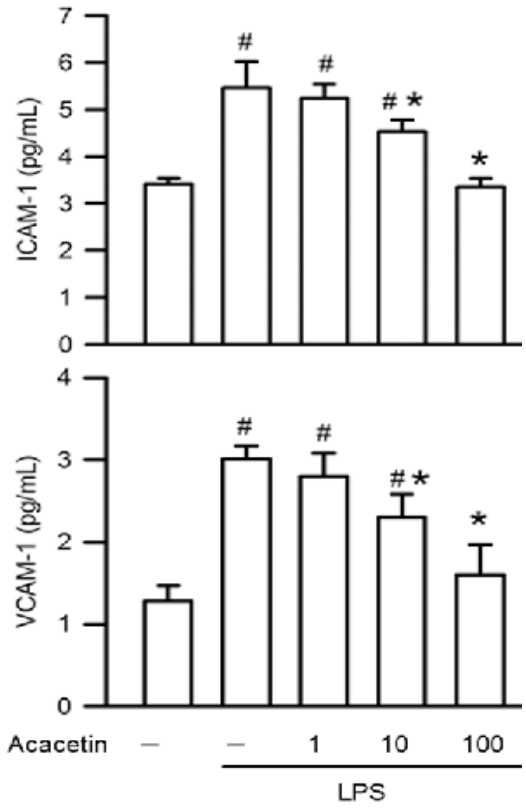


Fig 3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VCAM-1 (pg/mL)
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—
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100
LPS

Fig 4

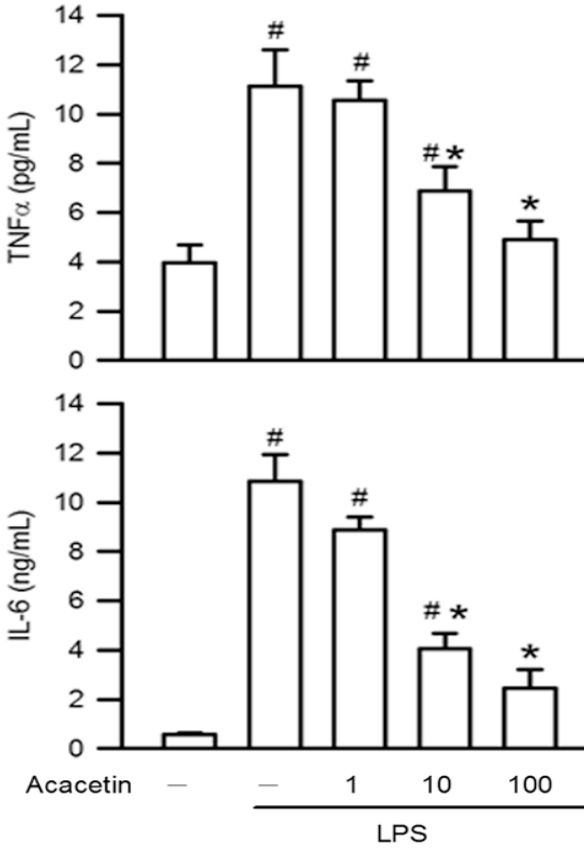
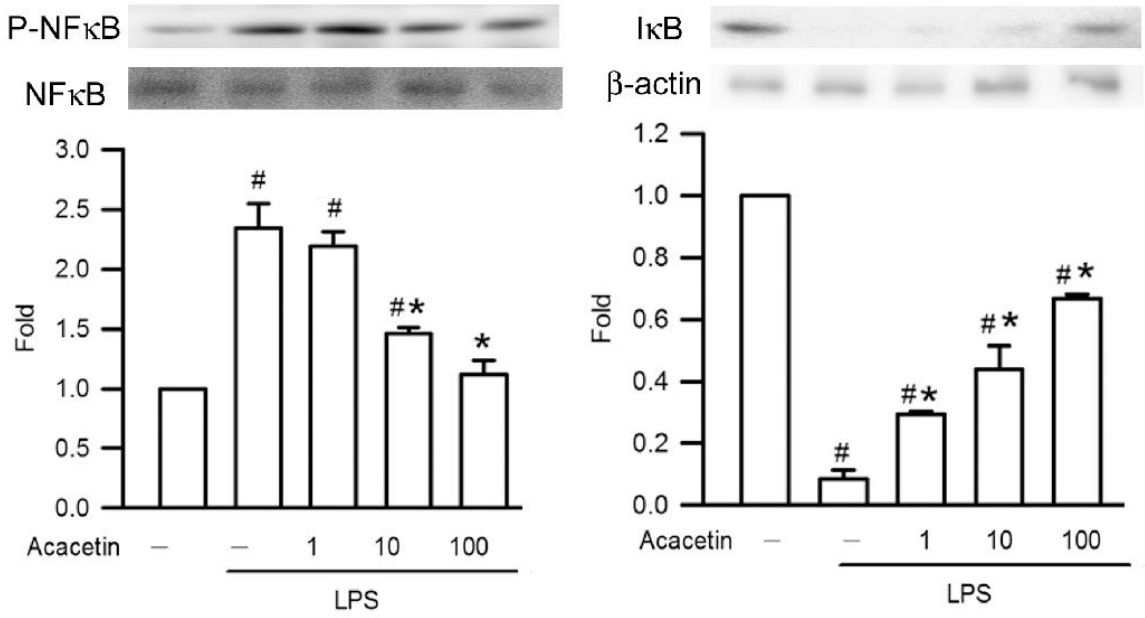

TNFα (pg/mL)
IL-6 (ng/mL)
Acacetin
—
—
1
10
100
LPS

Fig 5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NFκB
IκB
β-actin
Fold
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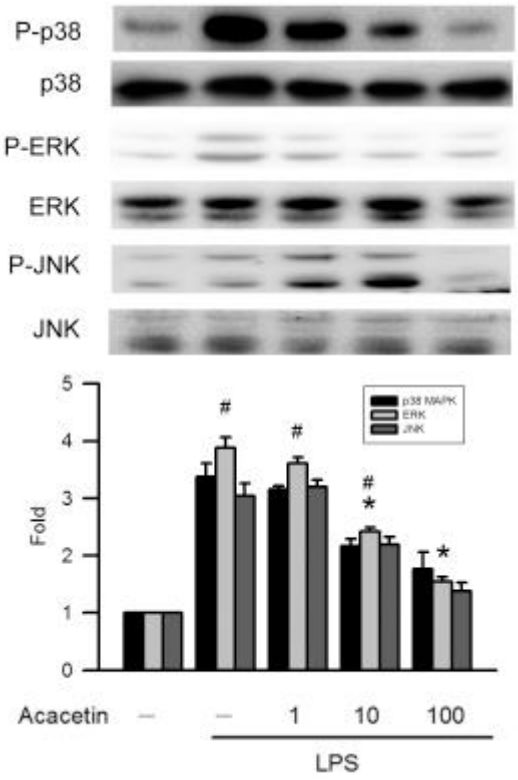
2.0
1.5
1.0
0.5
0.0
1.2
1.0
0.8
0.6
0.4
0.2
0.0
#
#*
*
Acacetin
–
1
10
100
LPS

Fig 6


P-p38
p38
P-ERK
ERK
P-JNK
JNK
Fold
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4
3
2
1
0
#
#*
*
Acacetin
–
1
10
100
LPS

Fig 7

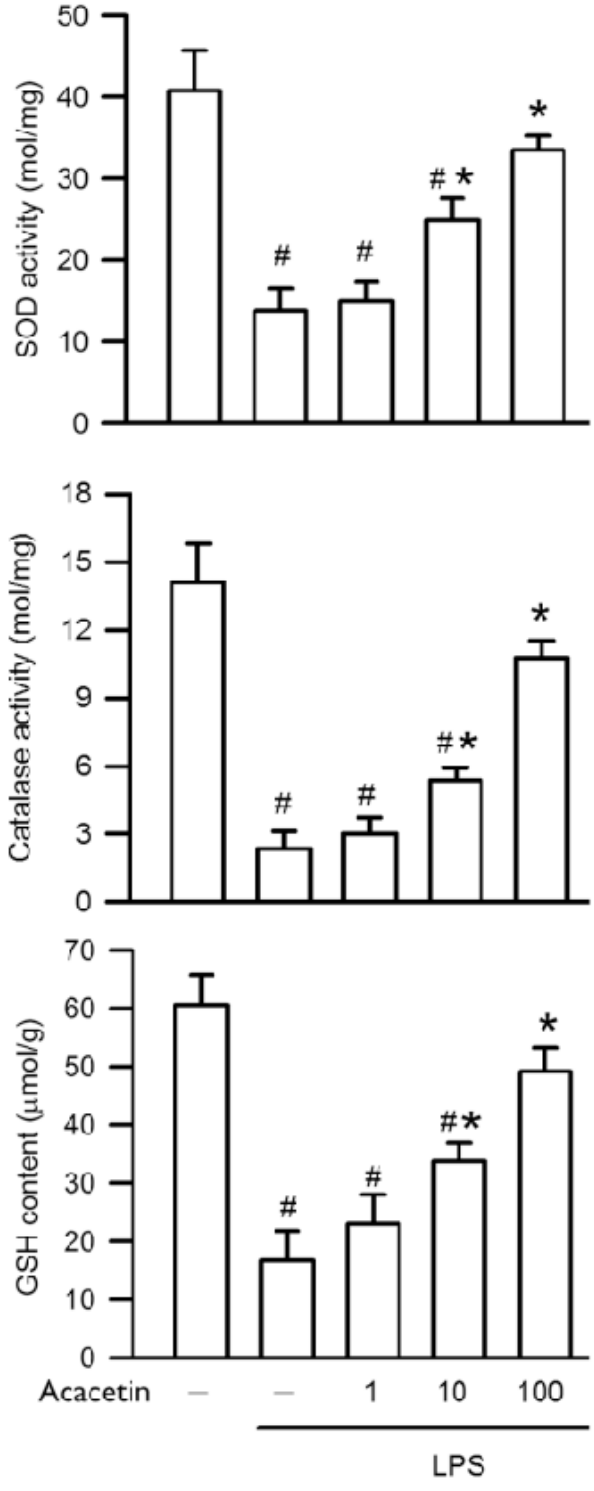
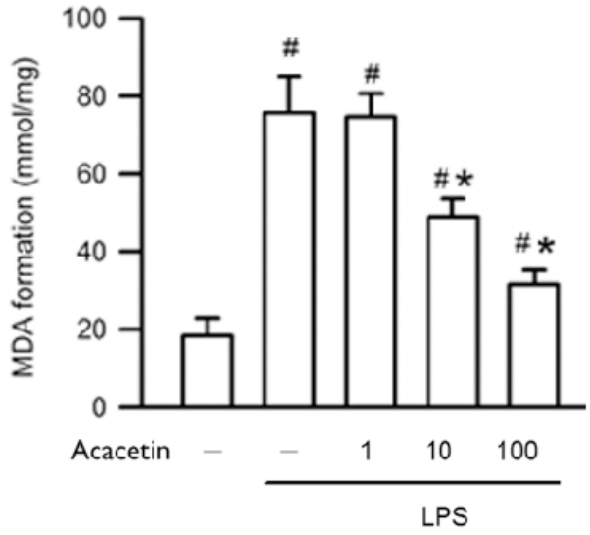

SOD activity (mol/mg)
50
40
30
20
10
0
#
#
#*
*
Catalase activity (mol/mg)
18
15
12
9
6
3
0
#
#
#*
*
GSH content (μmol/g)
70
60
50
40
30
20
10
0
#
#
#*
*
Acacetin
–
–
1
10
100
LPS

Fig 8



科技部補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否有嚴重損及公共利益之發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□ 未達成目標（請說明，以 100 字為限）

□ 實驗失敗

□ 因故實驗中斷

□ 其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 □未發表之文稿 ■撰寫中 □無

專利：□已獲得 □申請中 □無

技轉：□已技轉 □洽談中 □無

其他：（以 100 字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性），如已有嚴重損及公共利益之發現，請簡述可能損及之相關程度（以 500 字為限）

藉由本研究計畫，將使參與研究計畫的各式研究人員，包括其他見習人員對於急性肺損傷的致病機轉與治療方法，有著更深入的瞭解與認識。並能研究發刺槐素保護急性肺損傷的機轉及降低嗜中性球活化機制，作為臨床人體實驗的基礎。進一步開發更多的結構相近的衍生物，以期開發得到一最有效的藥物結構。並期待發表國際知名期刊，以提升台灣於世界營養保健領域的地位。

科技部補助專題研究計畫成果彙整表

計畫主持人：關宇翔	計畫編號：MOST 104－ 2320 － B － 040 － 006 －
計畫名稱：研究刺槐素的急性肺損傷保護機制	

成果項目					量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文				篇	請附期刊資訊。
		研討會論文			1		Li MC, Wu WJ, Kuan YH. (2015, Mar). The effects of DHMPC on the interaction between endothelial cells and macrophages. Annual Conference of Biomedical Sciences. (Taipei, Taiwan)P617
		專書				本	請附專書資訊。
		專書論文				章	請附專書論文資訊。
		技術報告				篇	
		其他				篇	
	智慧財產權及成果	專利權	發明專利	申請中		件	請附佐證資料，如申請案號。
				已獲得			請附佐證資料，如獲證案號。
			新型/設計專利				
		商標權					
		營業秘密					
		積體電路電路布局權					
		著作權					
		品種權					
		其他					
	技術移轉	件數				件	
		收入				千元	1. 依「科技部科學技術研究發展成果歸屬及運用辦法」第 2 條規定，研發成果收入係指執行研究發展之單位因管理及運用研發成果所獲得之授權金、權利金、價金、股權或其他權益。 2. 請註明合約金額。

國外	學術性論文	期刊論文				請附期刊資訊。
		研討會論文			1	篇	Kuan YH, Chen WY, Chen CJ, Yang ML, Lee SS, Lee CY, Ho YC, Liang CH. (2016, May) Protective effect of acacetin on lipopolysaccharide-induced acute lung injury via reduction of p38 MAPK and JNK phosphorylation. The 19th International Congress of Cytology: ICC2016. (Yokohama, Japan). P-087.
		專書				本	請附專書資訊。
		專書論文				章	請附專書論文資訊。
		技術報告				篇	
		其他				篇	
	智慧財產權及成果	專利權	發明專利	申請中		件	請附佐證資料，如申請案號。
				已獲得			請附佐證資料，如獲證案號。
			新型/設計專利				
		商標權					
		營業秘密					
		積體電路電路布局權					
		著作權					
		品種權					
		其他					
	技術移轉	件數				件	
		收入				千元	1. 依「科技部科學技術研究發展成果歸屬及運用辦法」第 2 條規定，研發成果收入係指執行研究發展之單位因管理及運用研發成果所獲得之授權金、權利金、價金、股權或其他權益。 2. 請註明合約金額。
	本國籍	大專生				人次	

參與計畫人力		碩士生			
		博士生			
		博士後研究員			
		專任助理			
	非本國籍	大專生			
		碩士生			
		博士生			
		博士後研究員			
		專任助理			
其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)					

科技部補助專題研究計畫出席國際學術會議心得報告

日期：105 年 10 月 20 日

計畫編號	MOST 104－ 2320 － B － 040 － 006 －		
計畫名稱	研究刺槐素的急性肺損傷保護機制		
出國人員姓名	關宇翔	服務機構及職稱	中山醫學大學醫學系藥理學科
會議時間	2016 年 5 月 28 日至 2016 年 6 月 1 日	會議地點	日本橫濱
會議名稱	(中文) 第十九屆國際細胞學會大會 (英文) The 19 th International Congress of Cytology		
發表題目	(中文) 刺槐素經由降低 p38 MAPK 與 JNK 磷酸化，進而保護由脂多醣誘發之急性肺損傷 (英文) Protective effect of acacetin on lipopolysaccharide -induced acute lung injury via reduction f p38 MAPK and JNK phosphorylation		

一、參加會議經過

本人於 2016 年 5 月 27 日至日本成田機場，入境日本。隔日 2016 年 5 月 27 日抵達日本橫濱國際平和會場 (Pacifico Yokohama) 進行為期五天的第十九屆國際細胞學會大會。於 28 當日，即參觀 IAC cytotechnology Exmination 議程。於 29 日，則參與 MOLECULAR TESTING IN CYTOLOGY FOR LUNG CANCER 與 NEW TECHNOLOGY AND MOLECULAR CYTOLOGY 兩場研討會。30 至 31 日則參與海

報論文發表與觀摩 (如下圖)，與參與了 MOLECULAR CYTOLOGY - NOVEL STRATEGIES FOR CANCER CONTROL 研討會。至 6 月 1 日，則參與 VALUE OF CYTOLOGICAL DIAGNOSIS FOR MALT LYMPHOMA: A DIAGNOSTIC CHALLENGE 研討會，隔日返國。

二、與會心得

由於第一次參與的國外國際型會議，卻實令本人有著相當高的期許。而到了會場後，果然發現大型國際會議的專業性與多元性。當然，在參與各式研討會與參觀各式海報論文的過程中，也令我學習到許多新的知識與觀念，如新的細胞染色與顯微觀察模式、肺癌分子醫學與檢驗方面的新知、淋巴癌發生的分子機制與醫學檢驗方面的新觀念。在在令人驚訝與向往學習。也更為期待，下次的國際會議。

三、發表論文全文或摘要

Protective effect of acacetin on lipopolysaccharide-induced acute lung injury via reduction f p38 MAPK and JNK phosphorylation

Yu- Hsiang Kuan[1,2], Wen- Ying Chen[3], Chun- Jung Chen[3], Ming- Ling Yang[4], Shiuan- Shinn Lee[5], Chien-Ying Lee[1,2], Yung- Chyuan Ho[6], Ching- Hui Liang[1,2]

[1]Department of Pharmacology, Chung Shan Medical University, [2]Department of Pharmacy, Chung Shan Medical University Hospital, [3]Department of Veterinary Medicine, National Chung Hsing University, [4]Department of Anatomy, School of Medicine, Chung Shan Medical University, [5]School of Public Health, Chung Shan Medical University, [6]School of Medical Applied Chemistry, Chung Shan Medical University

Background: Acute lung injury (ALI), the serious and acute pulmonary inflammatory disorder, remains the high incidence and mortality in patients. Up to now, there are no effective therapy strategies available clinically for the improvement of ALI. Acacetin, belonging to the family of flavonoids, is present in a vast of plants, such as *Saussurea involucrate*, *Cirisium rhinoceros*, *Clerodendrum inerme* (L.) Gaertn, Compositae. Acacetin has been shown to cause beneficial effects against inflammation -related diseases such as inflammatory pain, Parkinson's disease, asthma, and cancer. The aim of this study to investigate the potential protective effects of acacetin and the molecular mechanisms involved in lipopolysaccharide (LPS)-induced ALI.

Materials and Methods: In the mice ICR model, ALI was induced by intratracheal administration of LPS, and acacetin at various concentrations (0, 1, 10, 100 μmol/kg) gwas intraperitoneal administration for 30 min prior to LPS treatment for 6 h. Leukocytes infiltration was measured by Giemsa stain. Activation of antioxidant enzymes, which including superoxide dismutase, catalase, and glutathione peroxidase, was determined by commercially assay kits. Expression and phosphorylation of proteins was measured by western blot.

Results: Pretreatment with acacetin inhibited leukocytes infiltration in lungs in LPS-induced ALI in a concentration-dependent manner (Fig 1A). As shown in figure 1B-D, decreased activities of superoxide dismutase, catalase, and glutathione peroxidase induced by LPS were reversed by acacetin in a dose-dependent manner. Phosphorylation of NF-κB and degradation of IκBa were inhibited by acacetin in LPS-induced ALI in a concentration-dependent manner (Fig 2 A-B). Phosphorylation of p38 and JNK were inhibited by acacetin in LPS-induced ALI in a concentration-dependent manner (Fig 2 C-D).

Figure 1. Effect of acacetin on neutrophil infiltration and antioxidant enzymes activities in LPS-induced ALI.

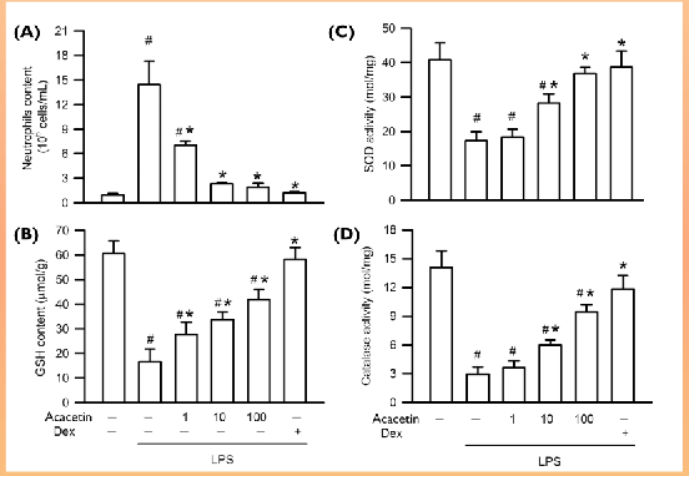
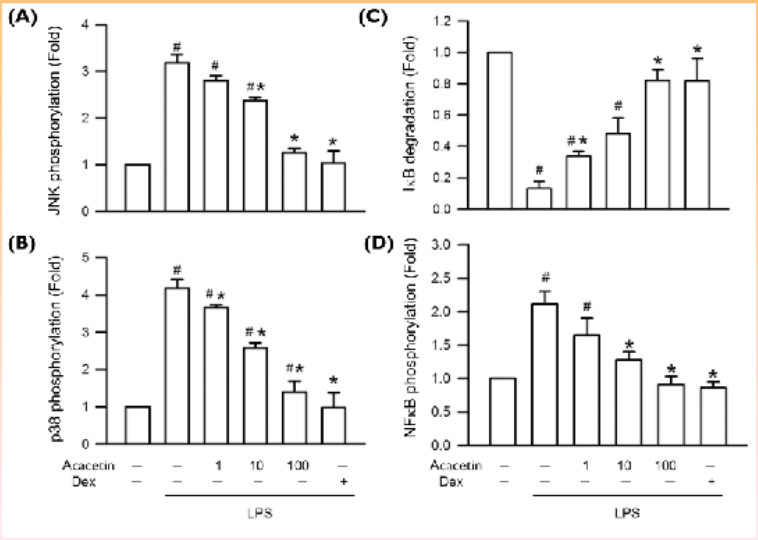


Figure 2. Effect of acacetin on p38, JNK, NFκB phosphorylation and IκB degradation



Conclusion: In conclusion, the protective mechanisms of acacetin are up-regulation of antioxidative enzymes and inhibition of NFκB phosphorylation via p38 and JNK phosphorylation in LPS-induced ALI. This study was supported by research grants from the Ministry of Science and Technology of Taiwan (MOST 104-2320-B-040-006-).

四、建議

謝謝科技部的支持，讓參與國際會議之行順利成行。

五、攜回資料名稱及內容

The 19 th International Congress of Cytology 大會議程。內容為本次會議議程與相關活動內容。

六、其他

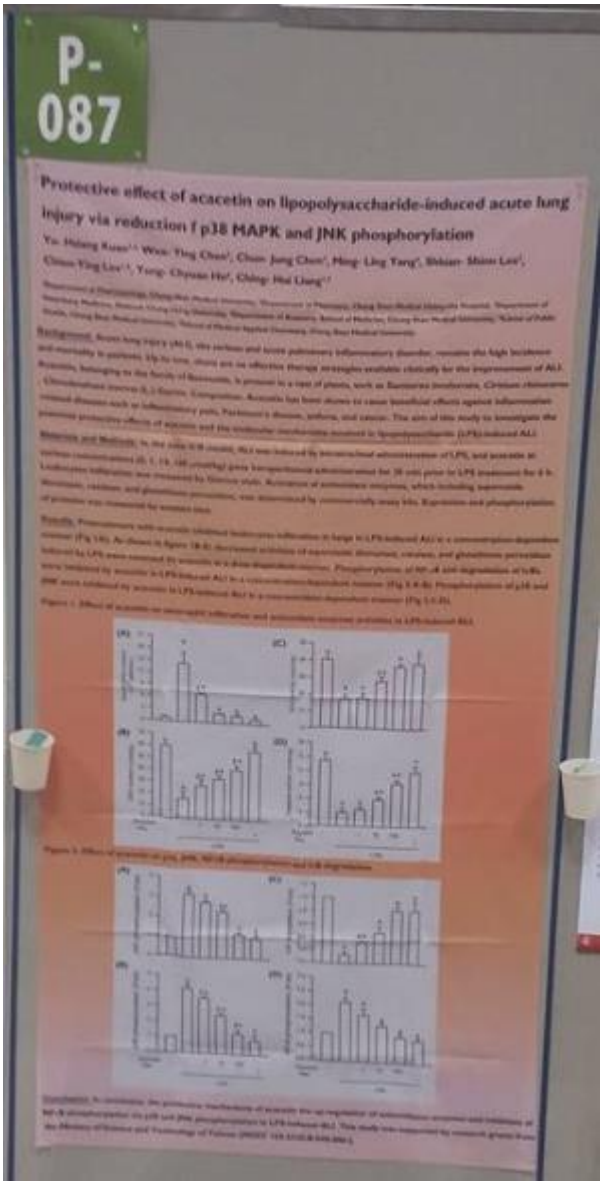
科技部補助專題研究計畫出席國際學術會議心得報告

日期：105 年 10 月 20 日

計畫編號	MOST 104－ 2320 － B － 040 － 006 －		
計畫名稱	研究刺槐素的急性肺損傷保護機制		
出國人員姓名	關宇翔	服務機構及職稱	中山醫學大學醫學系藥理學科
會議時間	2016 年 5 月 28 日至 2016 年 6 月 1 日	會議地點	日本橫濱
會議名稱	(中文) 第十九屆國際細胞學會大會 (英文) The 19 th International Congress of Cytology		
發表題目	(中文) 刺槐素經由降低 p38 MAPK 與 JNK 磷酸化，進而保護由脂多醣誘發之急性肺損傷 (英文) Protective effect of acacetin on lipopolysaccharide -induced acute lung injury via reduction f p38 MAPK and JNK phosphorylation		

一、參加會議經過

本人於 2016 年 5 月 27 日至日本成田機場，入境日本。隔日 2016 年 5 月 27 日抵達日本橫濱國際平和會場 (Pacifico Yokohama) 進行為期五天的第十九屆國際細胞學會大會。於 28 當日，即參觀 IAC cytotechnology Exmination 議程。於 29 日，則參與 MOLECULAR TESTING IN CYTOLOGY FOR LUNG CANCER 與 NEW TECHNOLOGY AND MOLECULAR CYTOLOGY 兩場研討會。30 至 31 日則參與海報論文發表與觀摩 (如下圖)，與參與了 MOLECULAR CYTOLOGY - NOVEL STRATEGIES FOR CANCER CONTROL 研討會。至 6 月 1 日，則參與 VALUE OF CYTOLOGICAL DIAGNOSIS FOR MALT LYMPHOMA: A DIAGNOSTIC CHALLENGE 研討會，隔日返國。



二、與會心得

由於第一次參與的國外國際型會議，卻實令本人有著相當高的期許。而到了會場後，果然發現大型國際會議的專業性與多元性。當然，在參與各式研討會與參觀各式海報論文的過程中，也令我學習到許多新的知識與觀念，如新的細胞染色與顯微觀察模式、肺癌分子醫學與檢驗方面的新知、淋巴癌發生的分子機制與醫學檢驗方面的新觀念。在在令人驚訝與向往學習。也更為期

待，下次的國際會議。

三、發表論文全文或摘要

Protective effect of acacetin on lipopolysaccharide-induced acute lung injury via reduction f p38 MAPK and JNK phosphorylation

Yu- Hsiang Kuan[1,2], Wen- Ying Chen[3], Chun- Jung Chen[3], Ming- Ling Yang[4], Shiuan- Shinn Lee[5], Chien-Ying Lee[1,2], Yung- Chyuan Ho[6], Ching- Hui Liang[1,2]

[1]Department of Pharmacology, Chung Shan Medical University, [2]Department of Pharmacy, Chung Shan Medical University Hospital, [3]Department of Veterinary Medicine, National Chung Hsing University, [4]Department of Anatomy, School of Medicine, Chung Shan Medical University, [5]School of Public Health, Chung Shan Medical University, [6]School of Medical Applied Chemistry, Chung Shan Medical University

Background: Acute lung injury (ALI), the serious and acute pulmonary inflammatory disorder, remains the high incidence and mortality in patients. Up to now, there are no effective therapy strategies available clinically for the improvement of ALI. Acacetin, belonging to the family of flavonoids, is present in a vast of plants, such as *Saussurea involucrate*, *Cirisium rhinoceros*, *Clerodendrum inerme* (L.) Gaertn, Compositae. Acacetin has been shown to cause beneficial effects against inflammation -related diseases such as inflammatory pain, Parkinson's disease, asthma, and cancer. The aim of this study to investigate the potential protective effects of acacetin and the molecular mechanisms involved in lipopolysaccharide (LPS)-induced ALI.

Materials and Methods: In the mice ICR model, ALI was induced by intratracheal administration of LPS, and acacetin at various concentrations (0, 1, 10, 100 μmol/kg) gwas intraperitoneal administration for 30 min prior to LPS treatment for 6 h. Leukocytes infiltration was measured by Giemsa stain. Activation of antioxidant enzymes, which including superoxide dismutase, catalase, and glutathione peroxidase, was determined by commercially assay kits. Expression and phosphorylation of proteins was measured by western blot.

Results: Pretreatment with acacetin inhibited leukocytes infiltration in lungs in LPS-induced ALI in a concentration-dependent manner (Fig 1A). As shown in figure 1B-D, decreased activities of superoxide dismutase, catalase, and glutathione peroxidase induced by LPS were reversed by acacetin in a dose-dependent manner. Phosphorylation of NF-κB and degradation of IκBa were inhibited by acacetin in LPS-induced ALI in a concentration-dependent manner (Fig 2 A-B). Phosphorylation of p38 and JNK were inhibited by acacetin in LPS-induced ALI in a concentration-dependent manner (Fig 2 C-D).

Figure 1. Effect of acacetin on neutrophil infiltration and antioxidant enzymes activities in LPS-induced ALI.

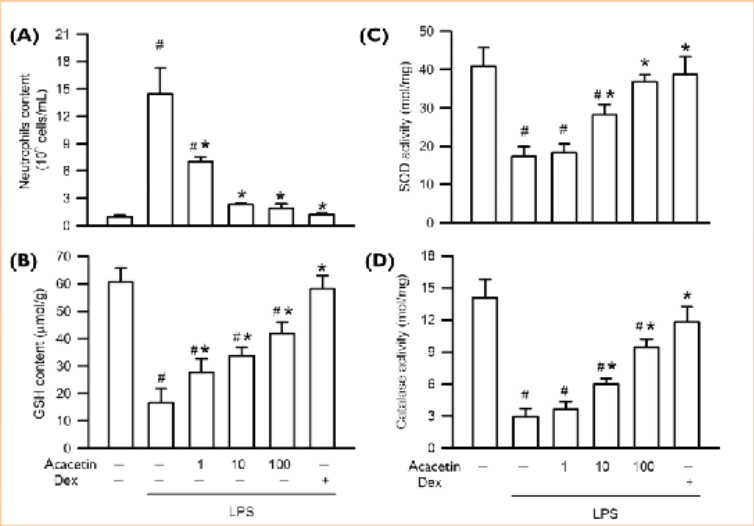
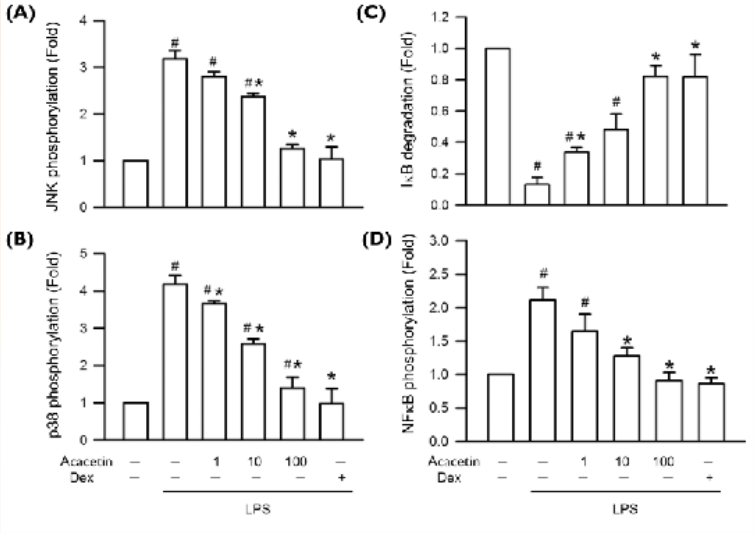


Figure 2. Effect of acacetin on p38, JNK, NFκB phosphorylation and IκB degradation



Conclusion: **In conclusion, the protective mechanisms of acacetin are up-regulation of antioxidative enzymes and inhibition of NFκB phosphorylation via p38 and JNK phosphorylation in LPS-induced ALI. This study was supported by research grants from the Ministry of Science and Technology of Taiwan (MOST 104-2320-B-040-006-).**

四、建議

謝謝科技部的支持，讓參與國際會議之行順利成行。

五、攜回資料名稱及內容

The 19 th International Congress of Cytology 大會議程。內容為本次會議議程與相關活動內容。

六、其他

科技部補助計畫衍生研發成果推廣資料表

日期:2016/10/19

科技部補助計畫	計畫名稱: 研究刺槐素的急性肺損傷保護機制
	計畫主持人: 關宇翔
	計畫編號: 104-2320-B-040-006- 學門領域: 營養保健

無研發成果推廣資料

104年度專題研究計畫成果彙整表

計畫主持人：關宇翔	計畫編號：104-2320-B-040-006-
計畫名稱：研究刺槐素的急性肺損傷保護機制	

成果項目					量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文			0	篇	
		研討會論文			1		Li MC, Wu WJ, Kuan YH. (2015, Mar). The effects of DHMPC on the interaction between endothelial cells and macrophages. Annual Conference of Biomedical Sciences. (Taipei, Taiwan)P617
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
國外	學術性論文	期刊論文			0	篇	
		研討會論文			1		1.Kuan YH, Chen WY, Chen CJ, Yang ML, Lee SS, Lee CY, Ho YC, Liang CH. (2016, May) Protective effect of acacetin on lipopolysaccharide-induced acute lung injury via reduction of p38 MAPK and JNK phosphorylation. The 19th International Congress of Cytology: ICC2016. (Yokohama, Japan). P-087.
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	

		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
參與計畫人力	本國籍	大專生			3	人次	
		碩士生			2		
		博士生			0		
		博士後研究員			0		
		專任助理			0		
	非本國籍	大專生			0		
		碩士生			0		
		博士生			0		
		博士後研究員			0		
		專任助理			0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）							

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）
   論文：□已發表　□未發表之文稿　■撰寫中　□無
   專利：□已獲得　□申請中　■無
   技轉：□已技轉　□洽談中　■無
   其他：（以200字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性，以500字為限）
   藉由本研究計畫，將使參與研究計畫的各式研究人員，包括其他見習人員對於急性肺損傷的致病機轉與治療方法，有著更深入的瞭解與認識。並能研究發刺槐素保護急性肺損傷的機轉及降低嗜中性球活化機制，作為臨床人體實驗的基礎。進一步開發更多的結構相近的衍生物，以期開發得到一最有效的藥物結構。並期待發表國際知名期刊，以提升台灣於世界營養保健領域的地位。

4. 主要發現
   本研究具有政策應用參考價值：■否　□是，建議提供機關
   （勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
   本研究具影響公共利益之重大發現：■否　□是
   說明：（以150字為限）