科技部補助專題研究計畫成果報告
期末報告

探討球薑酮經由 PI3K 路徑降低嗜中性球活化進而預防細菌性急性肺損傷

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計 畫 主 持 人 ： 關宇翔
共 同 主 持 人 ： 陳春榮
計畫參與人員： 碩士班研究生-兼任助理人員：曾璟淇
碩士班研究生-兼任助理人員：李妙卿
大專生-兼任助理人員：梁清惠
大專生-兼任助理人員：彭亞婷
大專生-兼任助理人員：周文安

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中 華 民 國 104 年 10 月 13 日

中文摘要： 衛生福利部的調查報告指出，國人因肺炎相關疾病引發死亡案例約佔所有病因死亡率的40%。其中，急性肺損傷為常見且嚴重急症，可惡化成急性呼吸窘迫症候群，引發多重器官衰竭，增加死亡風險。細菌性感染則是誘發急性肺損傷的主因之一。急性肺損傷的組織病理變化為嗜中性球浸潤、肺血管滲透性增加、肺壁增厚、肺水腫、玻璃膜形成等。過程中，嗜中性球的浸潤活化扮演著關鍵角色。活化的嗜中性球會產生趨化、吞噬、去顆粒、呼吸爆發、胞外殺菌誘補網的作用，適度的嗜中性球活化可毒殺入侵細菌，但過度的活化則會傷害週邊正常組織，引發急性肺損傷及相關併發症。對於急性肺損傷的治療目前多以支持療法為主，並無有效的藥物可用。球薑酮（zerumbone）為薑科植物紅球薑的主成分之一。紅球薑為日常生活中常用的香料或調味料，於傳統醫學上主要用於祛風散寒、溫中止痛、肺寒咳嗽等作用。由本研究計畫發現，球薑酮有效降低了內毒素所引發的急性肺損傷的病理組織的變化、嗜中性球浸潤、肺水腫。進一步，更發現球薑酮可有效降低促發炎物質，如細胞激素、黏附分子、iNOS、COX-2。球薑酮主要經由 Akt- NF-κB 路徑，來降低內毒素所引發的急性肺損傷。

中文關鍵詞： 球薑酮； 急性肺損傷； 促發炎介質； NFκB； Akt

英文摘要： The morbidity and mortality rates of acute lung injury (ALI) still rank high among clinical illnesses. Lipopolysaccharide (LPS) induced sepsis is the major cause of ALI. Zerumbone has biological benefit effects, such as antioxidation, anti-inflammation, and neuroprotection. To study the potential protective effects and the mechanisms of zerumbone on LPS-induced ALI. ALI was induced in mice by intratracheal injection of LPS, and zerumbone at various concentrations was injected intraperitoneally 30 min prior to LPS. Pretreatment with zerumbone inhibited the histolopatholgical changes occur in lungs in LPS-induced ALI. Pretreatment with zerumbone not only inhibited leukocyte infiltration into bronchoalveolar lavage fluid (BALF) but also reduced the lung edema in LPS-induced ALI. Expression of adhesion molecules, proinflammatory cytokines, iNOS, and COX-2 induced by LPS was inhibited reversed by zerumbone. LPS-induced NF-κB phosphorylation, IκB degradation, and Akt phosphorylation were inhibited

by zerumbone. These results suggest that the protective mechanisms of zerumbone on endotoxin-induced ALI were via inhibition of Akt-NFκB activation.

英文關鍵詞： Zerumbone； acute lung injury； proinflammatory mediators； NFκB； Akt

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（□期中進度報告/■期末報告）

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共同主持人：陳春榮
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中　華　民　國 104 年 10 月 13 日

目錄……………………………………………………………………………………………1
中文摘要及關鍵詞………………………………………………………………………2
英文摘要及關鍵詞………………………………………………………………………2
前言……………………………………………………………………………………………3
研究方法……………………………………………………………………………………3
結果……………………………………………………………………………………………5
討論……………………………………………………………………………………………6
國科會補助專題研究計畫成果報告自評表……………………………………13

中文摘要

衛生福利部的調查報告指出，國人因肺炎相關疾病引發死亡案例約佔所有病因死亡率的40%。其中，急性肺損傷為常見且嚴重急症，可惡化成急性呼吸窘迫症候群，引發多重器官衰竭，增加死亡風險。細菌性感染則是誘發急性肺損傷的主因之一。急性肺損傷的組織病理變化為嗜中性球浸潤、肺血管滲透性增加、肺壁增厚、肺水腫、玻璃膜形成等。過程中，嗜中性球的浸潤活化扮演著關鍵角色。活化的嗜中性球會產生趨化、吞噬、去顆粒、呼吸爆發、胞外殺菌誘補網的作用，適度的嗜中性球活化可毒殺入侵細菌，但過度的活化則會傷害週邊正常組織，引發急性肺損傷及相關併發症。對於急性肺損傷的治療目前多以支持療法為主，並無有效的藥物可用。球薑酮 (zerumbone) 為薑科植物紅球薑的主成分之一。紅球薑為日常生活中常用的香料或調味料，於傳統醫學上主要用於祛風散寒、溫中止痛、肺寒咳嗽等作用。由本研究計畫發現，球薑酮有效降低了內毒素所引發的急性肺損傷的病理組織的變化、嗜中性球浸潤、肺水腫。進一步，更發現球薑酮可有效降低促發炎物質，如細胞激素、黏附分子、iNOS、COX-2。球薑酮主要經由 Akt- NF-κB 路徑，來降低內毒素所引發的急性肺損傷。

關鍵字

球薑酮; 急性肺損傷; 促發炎介質; NFκB; Akt

Abstract

The morbidity and mortality rates of acute lung injury (ALI) still rank high among clinical illnesses. Lipopolysaccharide (LPS) induced sepsis is the major cause of ALI. Zerumbone has biological benefit effects, such as antioxidation, anti-inflammation, and neuroprotection. To study the potential protective effects and the mechanisms of zerumbone on LPS-induced ALI. ALI was induced in mice by intratracheal injection of LPS, and zerumbone at various concentrations was injected intraperitoneally 30 min prior to LPS. Pretreatment with zerumbone inhibited the histolopatholgical changes occur in lungs in LPS-induced ALI. Pretreatment with zerumbone not only inhibited leukocyte infiltration into bronchoalveolar lavage fluid (BALF) but also reduced the lung edema in LPS-induced ALI. Expression of adhesion molecules, proinflammatory cytokines, iNOS, and COX-2 induced by LPS was inhibited reversed by zerumbone. LPS-induced NF-κB phosphorylation, IκB degradation, and Akt phosphorylation were inhibited by zerumbone. These results suggest that the protective mechanisms of zerumbone on endotoxin-induced ALI were via inhibition of Akt-NFκB activation.

Keywords

Zerumbone; acute lung injury; proinflammatory mediators; NFκB; Akt

.1. Introduction

Acute lung injury (ALI) is the acute pulmonary inflammatory injury and life-threatening disease. There are several histopathological features in ALI including alveolar neutrophil infiltration, alveolar wall thickness, edema, haemorrhage, and hyaline membrane formation. Lipopolysacchride (LPS), also called endotoxin and the major compoment of Gram-negative bacterial outer membranes, is the most important risk factor for ALI via multitude of pulmonary and extrapulmonary insults. After instillation of LPS into the lung induces activation of alveolar macrophages and epithelial cells which predominant cell types in the alveolar-capillary barrier. Among the proinflammatory mediators, including tumor necrosis factor (TNF)-α, interlukine (IL)-6, intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2), 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). Disruption of alveolar-capillary barrier is induced by LPS, which results in leakage of neutrophils and plasma protein into alveolar space and parenchyma. Nuclear factor (NF)-κB, a proinflammatory transcription factor, is one of the most important factor which participate in the reaultation of proinflammatory mediators generation. Phosphoinositide 3-kinase and its downstream effector, the protein kiase Akt, have been demonstrated to modulate NF-κB activation in LPS induced ALI.

Up to date, 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 deficiency and development. Zerumbone, chemical name: 2,6,9,9-tetramethyl-*(2E,6E,10E)*-cycloundeca-2,6,10-trien-1-one, is a cyclic eleven-membered sesquiterpene and the main component of the essential oil isolated from the wild ginger, *Zingiber zerumbet* Smith (Kitayama T, 2011; Prasannan et al., 2012). In mice, carrageenan- induced paw edema is inhibited by zerumbone (Sulaiman et al., 2010). Ultraviolet B radiation-induced inflammatory photokeratitis result in conrneal damage is protected by zerumbone (Chen et al., 2011). After overdosage of paracetamol administration, the inflammation and necrosis in the rat liver tissues are reduced zerumbone (Fakurazi et al., 2008). In murine RAW264.7 macrophage, zerumbone effectively inhibits LPS-induced TNF-α generation, production of nitrite and prostaglandin E2 which through iNOS and COX-2 respectively, via NF-κB activation (Murakami et al., 2005). Superoxide anion generation by phorbol easter is inhibited by zerumbone in differentiated HL-60 leukemia cells and Chinese hamster ovary-AS52 cells cells (Murakami et al., 2002). At present study, we aimed to determine the anti-inflammatory activity of zerumbone in ALI after LPS administration in animal model and to expose the mechanism involved.

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 HIF-1, IκB, phospo-p65, p65, iNOS, COX-2, 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 zerumbone at concentration of 0, 0.1, 1, or 10 μmol/kg or dexamethasone at concentration of 1 mg/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. Measurement of Lung edema

To evaluate LPS-induced edema of lungs was assessted by the wet to dry weight (W/D) ratio. After lungs excision, the wet weights were recorded. The lungs were placed in a dry ovean at 80°C for 24 hrs to obtain the dry weight. The W/D ratio was calculated to evaluate the edema of lungs.

2.5. Bronchoalveolar lavage and Cell Counting

After sacrifice, the lungs were lavaged with sterile saline and cell numbers were counted as described previously. Brifely, 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.

2.6. Measurement of cytokines and adhesion molecules expression.

The expression of cytokines, whcich 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.

2.7. 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 iNOS, COX-2, IκB, β-actin, phosphorylated and non-phosphorylated forms of p65 and Akt. After washed, the horseradish peroxidase-labeled IgG was added for 1 h, and the blots were developed using enhanced chemiluminescence.

2.8. 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 zerumbone on lung histopathology in LPS-induced ALI

To evaluate the effect of histopathological changes in the lungs after zerumbone administration on LPS-induced ALI, the mice pretreated with zerumbone 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 zerumbone pretreatment, as shown in Fig 1B, the lung specimens represented notably pathologic changes, including neutrophil infiltration, alveolar wall thickness, haemorrhage, and hyaline membrane formation. The LPS-induced pathologic changes were ameliorated by pretreatment with zerumbone 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 zerumbone has the ability to improvement the pathologic changs of the lungs in LPS-induced ALI murine model.

3.2. Effects of zerumbone on lung edema and leukocyte infiltration in LPS-induced ALI

Increased aveolar-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. Data had shown that lung edema was significantly increased after administration of LPS when compared to untreated control group ($p < 0.05$), while for mice pretreated with zerumbone at concentration of 10 μmol/kg, the lung edema was significantly reduced ($p < 0.05$) (Fig 2A). In addition, leukocyte infiltration was quantified by Giemsa stain. After LPS administration, extensive leukocyte infiltration into BALF was significantly increased, which compared to untreated control group ($p < 0.05$). Preretment with zerumbone, LPS induced leukocytes infiltration was inhibited in a concentration-dependent manner, significant inhibitory effect began at 1 μmol/kg ($p < 0.05$) (Fig 2B). These results indicated that the protective effect of zerumbone in LPS-induced ALI mice.

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

Adhesion molecules, ICAM-1 and VCAM-1, participate in the recrutitment 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 zerumbone significantly inhibited the expression

of ICAM-1 and VCAM-1 in a concentration-dependent manner with statistically significant inhibition from control at 1 and 10 μmol/kg, respectively ($p<0.05$) (Fig 3). These results indicated zerumbone reduced leukocyte infiltration in LPS-induced ALI via down-regulation of adhesion molecules expression.

3.4. Effects of zerumbone 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 zerumbone 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 zerumbone reduced LPS-induced ALI via down-regulation of TNF-α and IL-6 expression.

3.5. Effects of zerumbone on expression of iNOS and COX-2 in LPS-induced ALI

After LPS administration, the expression of iNOS and COX-2 was significantly increased in lungs ($p < 0.05$). However, pretreatment with zerumbone significantly inhibited the expression of iNOS and COX-2 in a concentration-dependent manner with statistically significant inhibition from control at 1 μmol/kg ($p<0.05$) (Fig 5). These results indicated zerumbone reduced LPS-induced ALI via down-regulation of iNOS and COX-2 expression.

3.6. Effects of zerumbone on the NFκB phosphorylation and IκB degradation in LPS-induced ALI

NFκB has a cirtical role in expression of adhesion molecules, proinflammatory cytokines, iNOS and COX-2 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 zerumbone reduced the NFκB phosphorylation in a concentration-dependent manner starting at 1 μmol/kg (p<0.05, Fig. 6A). In parallel with NFκB phosphorylation, the effect of zerumbone on IκB degradation was also investigated. Similar to NFκB phosphorylation, LPS induced degradation of IκB which was significantly inhibited by zerumbone in a concentration-dependent manner with a significant inhibitory effect also starting from 1 μmol/kg ($p<0.05$) (Fig 6B). These results indicated that zerumbone reduced LPS-induced expression of adhesion molecules, proinflammatory cytokines, iNOS and COX-2 by reducing the NFκB phosphorylation and IκB degradation.

3.7. Effects of zerumbone on the Akt phosphorylation in LPS-induced ALI

The evidence has demonstrated that Akt is the upstream factor in NFκB phosphorylation and IκB degradation in LPS-induced ALI. LPS stimulation significantly increased Akt phosphorylation in comparison to the control group (p < 0.05). Zerumbone inhibited LPS-induced phosphorylation of Akt in a concentration-dependent manner starting at 1 μmolg/kg ($p < 0.05$, Figure 7). These results indicated that zerumbone inhibited LPS-induced NFκB phosphorylation by reducing the Aktphosphorylation.

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.[2] 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.[26] 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 zerumbone improved the LPS-induced histopathological changes, lung edema, and leukocytes infiltration. These results indicated that zerumbone 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 zerumbone pretreatment when compared with the control group. These results indicated that zerumbone 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, and proinflammatory proteins, including iNOS and COX-2, which subsequently mediate the recruitment of PMNs, macrophages, and lymphocytes. In a murine model of LPS-induced ALI, zerumbone reduced the production of TNF-α, IL-6, iNOS, and COX-2 in BALF. The results indicate that zerumbone 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 cytokinesin 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 zerumbone in a concentration-dependent manner. We suggest that the mechanism of the protective effect of zerumbone on LPS-induced ALI was through the NFκB pathway. Akt 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 Akt phorphorylation in LPS-treated groups is prevented by zerumbone in a concentration-dependent manner. We suggest that the mechanism of the inhibition of NFκB pathway of zerumbone on LPS-induced ALI for the most part was through the Akt phosphorylation. The results gathered from the present study suggest that zerumbone could be an effective agent against endotoxin induced ALI.

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Figure legends

Figure 1 Effects of zerumbone pretreatment on histopathological changes of lung tissues in LPS-induced ALI (100X). (A) Control (B) LPS (C) 0.1 μmol/kg zerumbone + LPS (D) 1 μmol/kg zerumbone + LPS (E) 10 μmol/kg zerumbone + LPS (F) 1 mg/kg dexamethasone + LPS.

Figure 2 Effects of zerumbone on lung edema and leukocyte infiltration in LPS-induced ALI. (A) Lung edema determined by the W/D ratio. (B) 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 zerumbone on expression of adhesion molecules in LPS-induced ALI. Expression of adhesion molecules, including (A) ICAM-1 and (B)VCAM-1, in BALF was determimed 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, $p < 0.05$.

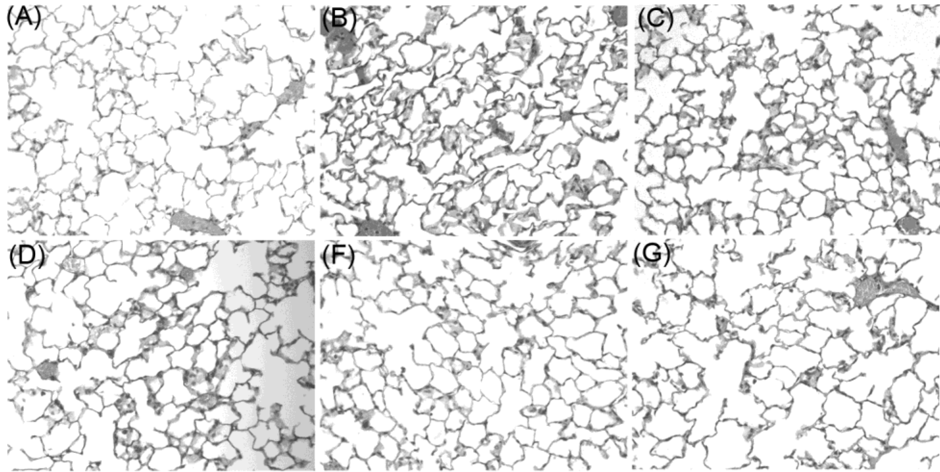
Figure 4 Effects of zerumbone on expression of proinflammatory cytokines in LPS-induced ALI. Expression of proinflammatory cytokines, including (A) TNF-α and (B) IL-6, in BALF was determimed by ELISA assay. Values are expressed as mean ± S.D. of 4 mice per group. # Represents significant difference between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

Figure 5 Effect of zerumbone on LPS-induced COX-2 and iNOS expression in the lungs. After the lungs harvested from post-treated animals were analyzed by Western blotting. The fold changes of iNOS and COX-2 expression 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 zerumbone 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 7 Effect of zerumbone on LPS-induced Akt phosphorylation in the lungs. After the lungs harvested from post-treated animals were analyzed by Western blotting. The fold changes of Akt 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 1



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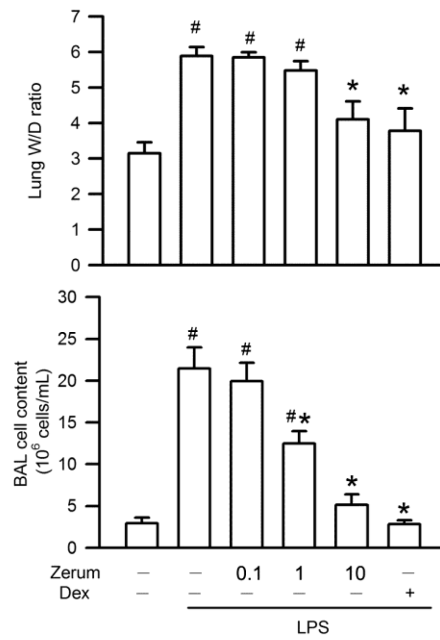
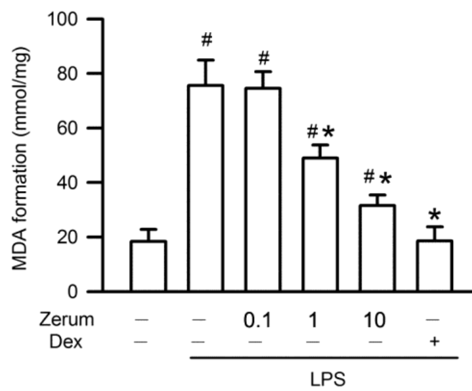


Figure 3



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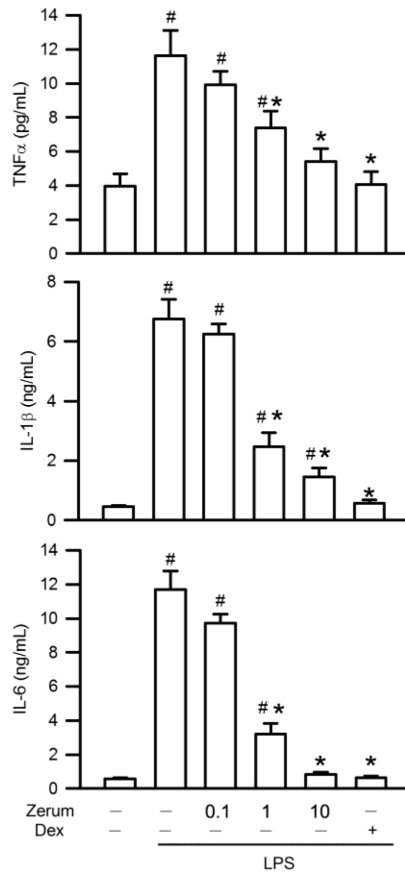

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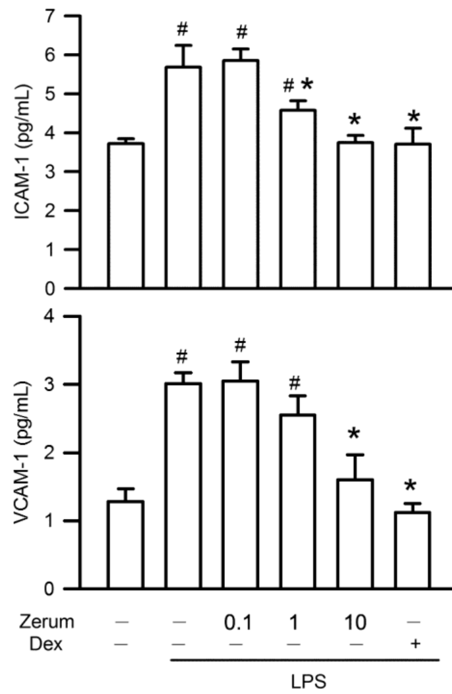

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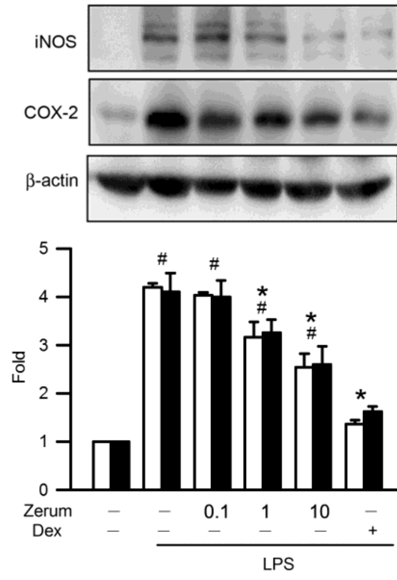

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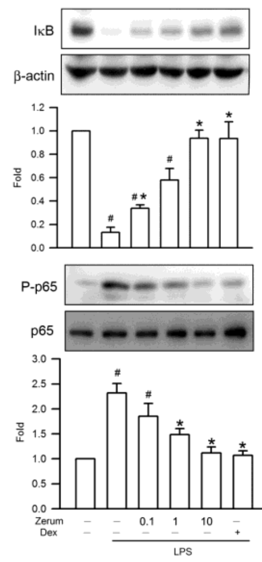

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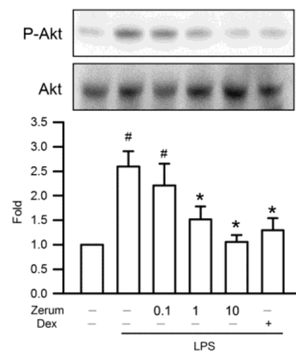

科技部補助專題研究計畫成果報告自評表

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）

1. 藉由此計畫，能增加對 ALI 機轉及治療方法，能有更深入的瞭解與認識。

2 瞭解球薑酮保護 ALI 的機轉及降低 PMN 活化機制，作為臨床人體實驗的基礎。

3. 期望球薑酮能成為一良好保健食品，以做為預防細菌感染所誘發的 ALI，而降低因疾病所造成的社會與經濟成本。

4. 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。

5 發表論文國際知名期刊，以提升台灣於世界生物與藥學的地位。

科技部補助計畫衍生研發成果推廣資料表

日期:2015/10/08

<table>
<tr><td rowspan="3">科技部補助計畫</td><td>計畫名稱: 探討球薑酮經由 PI3K 路徑降低嗜中性球活化進而預防細菌性急性肺損傷</td></tr>
<tr><td>計畫主持人: 關宇翔</td></tr>
<tr><td>計畫編號: 103-2320-B-040-008-　　　學門領域: 保健營養</td></tr>
<tr><td colspan="2">無研發成果推廣資料</td></tr>
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103 年度專題研究計畫研究成果彙整表

計畫主持人：關宇翔			計畫編號：103-2320-B-040-008-				
計畫名稱：探討球薑酮經由 PI3K 路徑降低嗜中性球活化進而預防細菌性急性肺損傷							
成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	1	1	100%		
		研討會論文	1	1	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	2	2	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

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。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

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技轉：□已技轉 □洽談中 ■無

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