科技部補助專題研究計畫報告

探討antrosterol與luteolin 對Fine Particulate Matter (PM2.5)誘發急性肺損傷引發炎症反應與氧化損傷的保護功效及機轉(第3年)

報 告 類 別 ： 精簡報告
計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 106-2320-B-040-023-MY3
執 行 期 間 ： 108年08月01日至109年07月31日
執 行 單 位 ： 中山醫學大學醫學系微生物及免疫學科

計 畫 主 持 人： 張元衍
共 同 主 持 人： 賴全裕、邱慧玲

計畫參與人員： 碩士級-專任助理：莊雅淇
碩士級-專任助理：尤韻婷

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

中 華 民 國 109 年 10 月 31 日

中 文 摘 要 ： 依據前人的研究發現，空氣污染粒子包括粒徑小於10微米(PM10)及2.5微米(PM 2.5)之粒子可藉由呼吸道進入我們人體循環系統，導致使全身性傷害，並顯示懸浮微粒會造成許多肺部傷害，其中更包括了發炎反應的發生。相關研究更證實促發炎物質過度堆積、巨噬細胞、嗜中性球活化、肺血管內皮障壁喪失等傷害機制會惡化急性肺損傷。然而迄今為止，PM2.5的免疫調節作用的潛在機制仍不清楚。因此本研究將以in vitro 與in vivo的方式來探討在暴露PM2.5的條件下， PM2.5對炎症反應的影響為何? 同時，本研究將以木犀草素(luteolin)來探討其是否具抑制PM2.5所誘發之炎症反應及其機轉為何?

第一年結果發現luteolin可以抑制由PM2.5活化的 JAK、STAT1、STAT3與NF-κB的表現，同時誘導HO-1的表現量上升，進而抑制炎症反應相關物質如: iNOS、COX-2與炎症相關的細胞激素。我們也利用PM2.5先處理RAW264.7細胞24小時後，再添加LPS處理24小時，結果發現PM2.5會增強LPS誘發IL-6與TNF-表現的能力及iNOS、COX-2與NFB的表現量增加，因此推測PM2.5會增強LPS誘發的炎症反應。

第二年結果發現， PM2.5對3株細胞(A549、NCI-H460與MRC-5)增生皆無顯著差異，且皆會誘發IL-8與ICAM-1的分泌量，具有劑量效應(dose - dependently)。也發現，PM2.5皆可促使各種細胞株之PI3K p110、pAK、pERK1/2的表現量增加，接著我們以H460細胞株來處理各種抑制劑來進行探討，結果發現LY294002、UO128與PD98059抑制劑可抑制PM2.5誘發IL-8與ICAM-1的分泌量，因此我們確認了PM2.5應可藉由 PI3K/AKT/ERK1/2的路徑來調控IL-8與ICAM-1的分泌量。而處理luteolin可經由抑制此訊息傳遞路徑，進而抑制IL-8與ICAM-1分泌量上升。

第三年結果發現，我們將小鼠先以鼻滴(nasal drop instillation)處理PM2.5 (100 mg/kg/days)，一週後再以鼻滴處理LPS來誘導肺損傷模式，並評估給予luteolin探討是否有保護效果及相關機制為何?結果顯示，處理luteolin可顯著減少支氣管肺泡灌洗液中neutrophils的數目，且明顯改善肺臟病理組織變化，同時也可減少MPO活性及提升GSH、catalase 與 superoxide dismutase的活性，也會抑制肺組織中iNOS及NFkB表現。

中 文 關 鍵 詞 ： 空氣污染、懸浮微粒、木犀草素、炎症反應、支氣管肺泡灌洗液、急性肺損傷

英 文 摘 要 ： Epidemiological studies have shown that exposure to ambient fine particulate matter (PM2.5) is associated with respiratory diseases. Lung inflammation is a central feature of many pulmonary diseases, which can be induced by PM2.5 exposure. Particulate matters (PMs) are major components of air pollution that damages lung cells. However, the mechanisms remain to be elucidated. Therefore, this study will use in vitro and in vivo methods to explore the effect of PM2.5 on inflammation under conditions of PM2.5 exposure? At the same time, we will use luteolin to explore whether it can inhibit the inflammatory response induced by PM2.5 and its mechanism.

In the first year study: Our goal is to explore the inflammatory response of different doses of PM2.5 to LPS stimulation in vitro. In PM2.5 exposure MH-S cells, luteolin potently inhibited the production of NO, iNOS, COX-2 and inflammatory cytokine production. Our data indicate that luteolin diminishes the proinflammatory mediators NO and the expression of their regulatory genes, iNOS and COX-2, in PM2.5 exposure MH-S cells by inhibiting STAT1/3 dependent NF-κB activation and inducing HO-1 expression. We pretreated RAW264.7 cells with PM2.5 for 24 hours, and then added LPS for 24 hours. The results showed that PM2.5 can enhance to induce pro-inflammatory cytokines (IL-6, and TNF-α) and the expression of iNOS, COX-2 and NFκB in RAW264.7 cells compared with LPS-treated alone groups. Hence, it is speculated that PM2.5 would enhance the inflammatory response induced by LPS.
In the second year study: Human NSCLC cell lines (A549 and NCI-H460) and normal Lung Cells (MRC-5) were exposure PM2.5 for 24 h and after using western blotting to analyze the expression of PI3K, p-Akt, and p-ERK1/2. ICAM-1 and IL-8 protein secretion levels were determined by ELISA, respectively. Moreover, exposing H460, A549 and MRC-5 cells to PM2.5 activated ERK, NFκB, and Akt. Pretreating H460 cells with PI3K/Akt inhibitor, mitogen-activated protein kinase inhibitor and ERK inhibitor significantly blocked PM2.5 -induced ICAM-1 and IL-8 production. Additionally, we found that Luteolin inhibits of IL-8 and ICAM-1secretion by inhibiting the PI3K, p-Akt, p-ERK1/2, and p-NFκB expression by PM2.5 exposure A549, H460 and MRC-5 cells.
In the third year study: In the experimental part of in vivo, we pretreated the mice with nasal drop instillation to PM2.5, and then treated LPS with nasal drop instillation one week later to induce lung injury model. At the same time, to evaluate whether the administration of luteolin has a protective effect and related mechanisms. The results showed that the PM2.5 +LPS group induced changes in lung pathological sections including: neutrophil infiltration in alveolar fluid and increased MPO activity, and decreased activity of GSH, catalase and SOD in lung tissue, IL-6 and TNF-α released into the BALF were higher than those in the LPS group alone. In addition to explore whether of luteolin to reduce the inflammatory response induced by PM2.5. The results showed that treatment of luteolin can significantly reduce the number of neutrophils in the BALF, and significantly improve the pathological changes of the lung.

英文關鍵詞：Air pollution, Particulate matters (PMs), luteolin, inflammatory response, bronchoalveolar lavage fluid (BALF),

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科技部補助專題研究計畫成果報告

（■期末精簡報告）

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計畫類別：V個別型計畫　□整合型計畫
計畫編號：MOST　106-2320-B-040-023-MY3
執行期間：2017 年 08 月 01 日至 2020 年 07 月 31 日

執行機構及系所：中山醫學大學 醫學系微免科

計畫主持人：張元衍
共同主持人：邱慧玲、賴全裕
計畫參與人員：尤韻婷、陳偲茜、葉睿軒、鄒尚錞

本計畫除繳交成果報告外，另含下列出國報告，共 2 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
□出國參訪及考察心得報告

中文摘要:

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因此，經由上述實驗可知luteolin具有抗炎作用，其作用可能是藉由改善肺組織的病理學變化、減少BALF 中之嗜中性白血球浸潤及抑制炎症相關細胞激素來降低其對PM2.5及LPS導致急性肺損傷的保護作用。

關鍵字: 空氣污染、懸浮微粒、木犀草素、炎症反應、支氣管肺泡灌洗液、急性肺損傷

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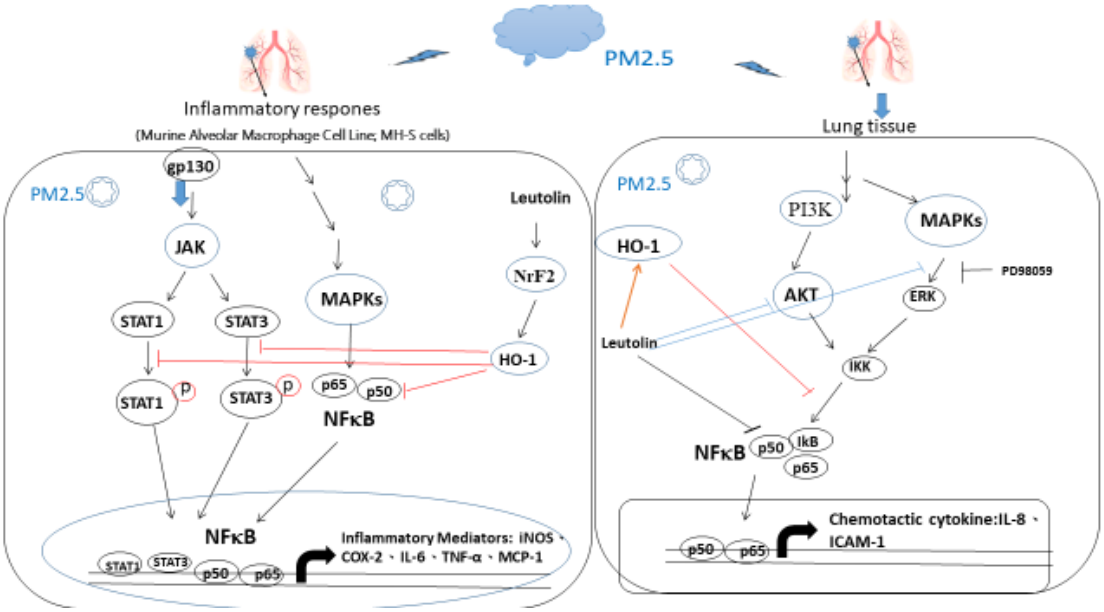
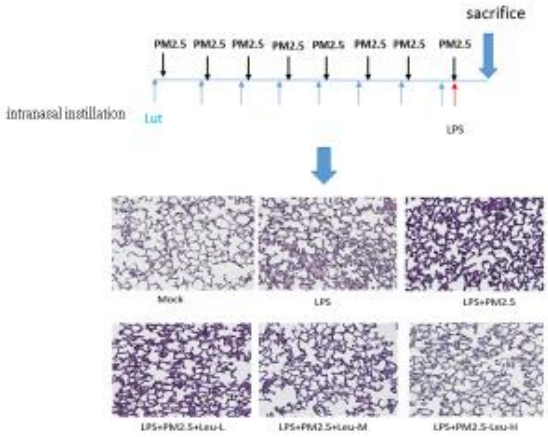
activity, and decreased activity of GSH, catalase and SOD in lung tissue, IL-6 and TNF-α released into the BALF were higher than those in the LPS group alone. In addition to explore whether of luteolin to reduce the inflammatory response induced by PM2.5. The results showed that treatment of luteolin can significantly reduce the number of neutrophils in the BALF, and significantly improve the pathological changes of the lung.

At the same time, it can also reduce the activity of MPO and increase the activity of GSH, catalase and superoxide dismutase, and also inhibit the expression of iNOS and NFκB in lung tissue. Therefore, our results confirmed that luteolin has anti-inflammatory effects, and its anti-inflammatory effects may be caused by improving the pathological changes of lung tissue, reducing the infiltration of neutrophils in BALF, and inhibiting inflammation-related cytokines to decrease acute lung injury induced by PM2.5 and LPS.

Key words: Air pollution, Particulate matters (PMs), luteolin, inflammatory response, bronchoalveolar lavage fluid (BALF), acute lung injury

結果:





The manifestations of pulmonary inflammation are as follows:

1. The results showed that the PM2.5 +LPS group induced changes in lung pathological sectionsincluding: neutrophil infiltration in alveolar fluid and increased MPO activity, and decreased activity of GSH, catalase and superoxide dismutase (SOD) in lung tissue, IL-6 and TNF-α released into the bronchoalveolar lavage fluid (BALF) were higher than those in the LPS group alone.
2. luteolin can significantly reduce the number of neutrophils in the BALF, and significantly improve the pathological changes of the lung. At the same time, it can also reduce the activity of MPO and increase the activity of GSH, catalase and superoxide dismutase, and also inhibit the expression of iNOS and NFκB in lung tissue.

本研究計畫證實 luteolin 可以藉由抑制 JAK/STAT3/ NF-κB，的路徑，進而抑制由 PM2.5 誘發 MHS 細胞的炎症反應；同時也發現 luteolin 可經由抑制 PM2.5 藉由 PI3K/AKT/ERK1/2 的路徑來調控 IL-8 與 ICAM-1 的分泌量。另外 luteolin 減緩小鼠處理 PM2.5 一週後再以鼻滴處理 Lipopolysaccharide (LPS)來誘導肺損傷模式中，可知 luteolin 可藉由改善肺組織的病理學變化、減少 BALF 中之中性粒細胞浸潤、提升抗氧化酵素活性及抑制炎症相關細胞激素來降低其對 PM2.5 及 LPS 導致急性肺損傷的保護作用。

106年度專題研究計畫成果彙整表

計畫主持人：張元衍	**計畫編號：**106-2320-B-040-023-MY3
計畫名稱：探討antrosterol與luteolin 對Fine Particulate Matter (PM2.5)誘發急性肺損傷引發炎症反應與氧化損傷的保護功效及機轉	

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	3		1.邱敬絜、張勁捷、廖靜瑜、林惠雯、張元衍。探討藻褐素(Fucoxanthin)對Fine Particulate Matter(PM2.5)誘發肺泡巨噬細胞(MH-S cells)引發炎症反應的保護功。2018保健食品與肌少症、惡病質及衰弱症之國際研討會。 2.林佳怡、黃安瀅、林惠雯、張元衍。探討漆黃素(Fisetin)對病毒感染肺泡巨噬細胞(MH-Scells)引發炎症反應的保護功效及機轉。2018保健食品與肌少症、惡病質及衰弱症之國際研討會。 韻婷、林惠雯、張元衍。 3. Luteolin inhibits the production of PM2.5-induced ICAM-1 expression in H460 cells via Akt, p38, and NFkB pathway. 2019 台灣食品科學技術學會第49 次會員大會暨研討會
		專書	0	本	
		專書論文	0	章	
		技術報告	0	篇	
		其他	0	篇	
國外	學術性論文	期刊論文	0	篇	
		研討會論文	2		1.Hui-Wen Lin, Yu-Hsiang Kuan, Yuan-Yen Chang. To investigate the protective effect of fisetinon PM2.5-induced the inflammatory responses and its mechanism in murine microglia cells. The 42nd 2.Yuan Yen Chang, Yi Chen Chen, Chin Lin Hsu, Hui-Wen Lin. Fucoxanthin inhibits PM2.5-induced inflammatory response in MH-S cells. 18th World Congress of Basic and Clinical Pharmacology (WCP). 2018. 07/01-07/06. Kyoto International Conference Center, Kyoto, Japan.
		專書	0	本	

		專書論文	1	章	2019. Nickel-induced VEGF expression via regulation of Akt, ERK1/2, NFκB, and AMPK pathways in H460 cells. Environ Toxicol. DOI: 10.1002/tox.22731 * co-correspondence
		技術報告	0	篇	
		其他	0	篇	
參與計畫人力	本國籍	大專生	0	人次	
		碩士生	0		
		博士生	0		
		博士級研究人員	0		
		專任人員	0		
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士級研究人員	0		
		專任人員	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。）					