行政院國家科學委員會補助專題研究計畫成果報告

細粒徑大氣微粒暴露對於肺部氧化傷害之修飾因子:敏感性呼吸道、老化，以及臭氧共同暴露

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中文摘要

大氣微粒 (particulate matter [PM]) 的增加與易感受性的老年族群之疾病率、甚至死亡率具有統計相關；然而微粒暴露與健康效應的機轉，目前仍不清楚。此外，內毒素 (endotoxin) 可以引發細胞在隨後的刺激下，產生更大的反應；因此，我們選擇老化及年輕大鼠來比較在單獨暴露於內毒素，或者合併之大氣微粒暴露後之肺部發炎反應的差異。粒徑小於 10 ìm 之大氣微粒是以石英纖維濾紙加以收集；年輕 (年紀 20 週大) 及老化 (年紀 60 週大) 之雄性大鼠，被區分為為以下六組：第一組為以 PBS 處理的未暴露組；第二組為暴露粗粒徑微粒；第三組為暴露細粒徑微粒；第四組為以多醣體 (lipopolysaccharide [LPS]) 內毒素引發；第五組為以多醣體內毒素引發後接續暴露於細微粒。暴露後，每組三隻年輕及老化的動物被麻醉並灌洗；而細胞激素 interleukin (IL)-6 及腫瘤壞死因子 tumor necrosis factor (TNF)-α濃度也以 ELISA 試劑組決定，來當作肺部灌洗細胞之發炎參數。單獨暴露於細粒徑微粒的老化大鼠相較於年輕大鼠，呈現顯著較高的 IL-6 反應 ($P < 0.05$, ANOVA with Tukey test)；單獨暴露於 LPS 的老化大鼠相較於年輕大鼠，也呈現顯著較高的 IL-6 及 TNF-α表現 ($Ps < 0.05$)。此外，老化大鼠同時暴露於 LPS 與細粒徑微粒對於 IL-6 的產生具有顯著的交互作用 ($P < 0.0001$)；然而，並沒有類似的 IL-6 表現在年輕大鼠中。當以 TNF-á 取代 IL-6 於統計分析時，年輕及老化大鼠同時暴露於 LPS 與細粒徑微粒對於 TNF-á 的產生，也呈現具有顯著的交互作用。在本研究中，單獨暴露於細粒徑微粒的老化大鼠相較於年輕大鼠，具有較高的肺部發炎反應。

關鍵字：大氣微粒，內毒素，老化動物，細胞激素 interleukin (IL)-6，腫瘤壞死因子 tumor necrosis factor (TNF)-α

Abstract

Increased ambient particulate matter (PM) associated with morbidity even mortality for susceptible elders. The mechanism of ambient PM exposure and health effect remains indistinct however. Additionally, endotoxin can prime target cells so that they show a greater response to a subsequent stimulus, therefore, we selected young and old rats to compare the differences of pulmonary inflammatory response after exposure to endotoxin alone or in combination with particles. Atmospheric particulates with diameters less than 10 ì m were collected on quartz fiber filters. Both young (20 weeks of age) and old (60 weeks of age) male rats were divided into five groups as follows: group 1, sham-exposed controls with PBS treatment; group 2, exposed to coarse particles; group 3, exposed to fine particles; group 4, lipopolysaccharide (LPS)-primed; group 5, LPS-primed followed by fine particles exposure. After dosing, three young and old animals of each dosage group were exhaustively lavaged. The interleukin (IL)-6 and tumor necrosis factor (TNF)-α concentration were also determined with ELISA kits as and inflammatory parameters in lung lavaged cells. Old rats exposed to fine particle alone showed a significantly higher response in IL-6 compared with young rats ($P < 0.05$, ANOVA with Tukey test). Significantly higher IL-6 and TNF-α expressions were also found in old rats exposed to LPS alone than young rats ($Ps < 0.05$). Additionally, there was a significant interaction on IL-6 production between LPS and fine particle exposure ($P < 0.0001$) in old rats. However, there were no similar results for IL-6 expression in young rats. When IL-6 was replaced by TNF-α in the statistical analysis, the significant interactions between LPS and fine particle in either young or old rats were also revealed. In our study, old rats exposed to fine particle or LPS had higher pulmonary inflammatory responses than young rats.

Key words: particulate matter, endotoxin, aging animal, interleukin-6, tumor necrosis factor-α

Introduction

Ambient particulate matter (PM) varies in size and chemical composition. In epidemiological studies have showed an associated between PM and morbidity and mortality and had data on the levels of fine particles (less than 2.5 μm in aerodynamic diameter [PM2.5]), the level of those was found to correlate with the observed effects (1, 2). Some researchers have hypothesized that fine particles might be more toxic than coarse particles (PM10), although they constitute only small proportion of the mass of PM, these smaller particles are present in very high numbers, have greater total surface area than larger particles (3). Furthermore, the elderly was reported to be more susceptible than others to the effect of particulate pollutants (4, 5). However, a biological mechanism linking particle exposure and pathophysiologic effects has not been well established.

On the other hand, endotoxin can prime target cells so that they show a greater response to a subsequent stimulus (6). Infection by gram-negative bacteria has been identified in several studies as the cause for pneumonia (7) and bronchitis (8), and epidemiological studies have identified pneumonia of the elderly as one factor for increased susceptibility to urban particles (9, 10). We therefore select young and old rats to compare the differences of pulmonary inflammatory response after exposure to endotoxin alone or in combination with particles.

Materials and methods

In this study, one site closed to the air quality monitoring stations in the Taichung area, central Taiwan, were selected for sampling. Ambient particulates with diameters less than 10 ì m were collected on quartz fiber filters using high volume samplers. Filters were suspended in phosphate-buffered saline (1x PBS). The weight of particle was measured by weighing the filters before and after a run. The concentration of particulates was determined by comparative the difference of the filters weight.

Male Fischer 344 rats, used in this study were purchased as specific pathogen-free animals. Both young (20 weeks of age) and old (60 weeks of age) male rats, were divided into five groups as follows (three young and three old rats per group): group 1, sham-exposed controls with PBS treatment; group 2, exposed to coarse particles, group 3, exposed to fine particles, group 4, lipopolysaccharide (LPS)-primed, group 5, LPS-primed followed by fine particles exposure. The intratracheal instillations were performed under halothane anesthesia by inserting a Teflon-tipped cannula into the trachea and delivering the ambient particles suspended in 0.25 ml saline. After dosing, three young and old animals of each dosage group were exhaustively lavaged.

The interleukin (IL)-6 and tumor necrosis factor (TNF)-α concentration were determined as cellular and inflammatory lung lavage parameters. Lavaged supernatants from rats were used for the determination of IL-6 and TNF-α levels with ELISA kits.

Results

Old rats exposed to the fine particle and LPS alone showed a significantly higher response in IL-6 compared with young rats ($Ps < 0.05$, ANOVA with Tukey test). Besides, the old animals exposed to LPS combined with fine particle also responded with a statistically significant increase in the IL-6 compared with young ones ($P < 0.05$). However, the elevated IL-6 protein production didn't be observed for old rats in control groups and with coarse particle exposure than young ones. When IL-6 was replace by TNF-α in the statistical analysis, an elevated TNF-α expression wasn't observed in old rats compared to young rats, as they were exposing to same conditions, except LPS exposure alone ($P < 0.05$).

Furthermore, the results of the analysis of the transformed IL-6 data shown there was a significant interaction between LPS and fine particle ($P < 0.0001$), and the main effects of fine particle ($P = 0.0001$) and LPS ($P < 0.0001$) in old rats. However, in fact, the presence of both LPS and fine particle

produced a lower response level than would be expected from data on each factor separately. On the other hand, there were no similar results for IL-6 expression in young rats. Subsequently, when IL-6 was replaced by TNF-α in the statistical analysis, the significant results in either young or old rats were revealed. There was a significant interaction between LPS and fine particle ($P < 0.0001$), and the main effects of fine particle ($P < 0.0001$) and LPS ($P < 0.0001$) in old or young rats. Similarly, the effect of fine particle for TNF-α expression in the old and young rats depended on the presence of LPS.

Discussion

The results revealed that old rats exposed to fine particle or LPS had higher pulmonary inflammatory responses than young rats.

Previous epidemiological studies indicated that susceptible elderly subjects are at an increased risk to be adversely affected by particulate pollution (4, 5) or LPS attack (9, 10). However, a biological mechanism linking particle exposure and pathophysiologic effects has not been well established. Thus, assessing the effects of particle size in aging animal models is critical to understanding how such pollutants may exert adverse effect. Here, we have demonstrated that increased pulmonary inflammation response by particulate and endotoxin can be detected in aged animals than others. Conceivably, the underlying mechanisms by which high particle burdens induce adverse effects in the young organisms are the same as those by which lower particles doses induce adverse effects in the susceptible aged organisms. However, significant changes occur with old age that can interfere with mechanistic pathways of injury of antioxidants in the lung (11), and reduced macrophage response to interferon-γ (12). Several inhalation studies of oxidant gases in rodent have also demonstrated an increased sensitivity of the aged organism compared with the young organism with respect to oxidant-induced cytokine response (13, 14).

It is interesting to observe that the variation of pulmonary cellular response by particle size. Particularly, this kind of association has not been clearly reported in healthy old and young animals. Although some researchers have considered that fine particles might be more toxic than coarse particles (PM10), since these smaller particles are present in very high numbers, have greater total surface area than larger particles under equal mass (3). Additionally, fine particles can contain numerous other compounds including transition metals (for example, iron and vanadium), which can potentially amplify effects in lung (15). The importance of these constitute compounds needs to be evaluated. However, a slight difference of pulmonary cytokine production by fine and coarse particles was observed in healthy old rats, not in young rats in this study.

Since epidemiological studies on particulate pollution showed adverse effects only in compromised persons, it is desired to use respective animal models to mimic specific human disorders. One such model involves inhalation of endotoxin (LPS) to induce and mimic early stages of respiratory tract infection with gram negative bacteria. Endotoxin exposure is known to result in inflammation and oxidative stress with the induction of inflammatory cytokines. However, at different time-point after endotoxin exposure, either increased sensitivity of target cells (priming, very early after exposure) or development of tolerance (one day or more after exposure) to a second stimulus can occur (16). Thus, we were unable to find the increased response of pulmonary inflammation by co-exposure to LPS and particulate compared to those by LPS or particulate exposure alone either in young or old rats. .

It appears that the aged organism is at a higher risk of lung inflammation. However, healthy rats, either young or old, did not show significant effects in our study after exposure to particle with the combination of LPS. In future, using environmentally relevant concentrations and resulting lung doses, appropriate animal models need to be

employed.

References

1. Dockery DW. Pope CA. Xu X. et al. An association between air pollution and mortality in six U.S. cities. New Engl J Med. 329:1753-9, 1993.
2. Pope CA 3rd. Thun MJ. Namboodiri MM. et al. Particulate air pollution as a predictor of mortality in a prospective study of U.S. adults. Am J Respir Crit Care Med. 151:669-74, 1995.
3. Peters A. Wichmann HE. Tuch T. et al. Respiratory effects are associated with the number of ultrafine particles. Am J Respir Crit Care Med. 155:1376-83, 1997.
4. Hwang JS. Chan CC. Effects of air pollution on daily clinic visits for lower respiratory tract illness. Am J Epidemiol. 155:1-10, 2002.
5. Schwartz J. Air pollution and hospital admissions for the elderly in Birmingham, Alabama. Am J Epidemiol. 139:589-98, 1994.
6. Khair OA. Davies RJ. Devalia JL. Bacterial-induced release of inflamematory mediators by bronchial epithelial cells. Eur Respir J. 9:1913-22, 1996.
7. Rello J. Rodriguez R. Jubert P. et al. Severe community-acquired pneumonia in the elderly: epidemiology and prognosis. Study Group for Severe Community-Acquired Pneumonia. Clin Infect Dis. 23:723-8, 1996.
8. Crome P. Bruce-Jones P. Infection in the elderly: studies with lomefloxacin. Am J Med. 92:126S-9S, 1992.
9. Moolgavkar SH. Luebeck EG. Anderson EL. Air pollution and hospital admissions for respiratory causes in Minneapolis-St. Paul and Birmingham. Epidemiology. 8:364-70, 1997.
10. Schwartz J. PM10, ozone, and hospital admissions for the elderly in Minneapolis-St. Paul, Minnesota. Arch Environ Health. 49:366-74, 1994.
11. Teramoto S. Fukuchi Y. Uejima Y. et al. Age-related changes in the antioxidant screen of the distal lung in mice. Lung. 172:223-30, 1994.
12. Hayakawa H. Sato A. Yagi T. et al. Superoxide generation by alveolar macrophages from aged rats: improvement by in vitro treatment with IFN-gamma. Mechanism Ageing Develop. 80:199-211, 1995.
13. Vincent R. Vu D. Hatch G. et al. Sensitivity of lungs of aging Fischer 344 rats to ozone: assessment by bronchoalveolar lavage. Am J Physiol. 271:L555-65, 1996.
14. Leeuwenburgh C. Hansen P. Shaish A. et al. Markers of protein oxidation by hydroxyl radical and reactive nitrogen species in tissues of aging rats. Am J Physiol. 274:R453-61, 1998.
15. Dye JA. Adler KB. Richards JH. et al. Epithelial injury induced by exposure to residual oil fly-ash particles: role of reactive oxygen species?. Am J Respir Cell Mol Biol. 17:625-33, 1997.
16. Parks DE. Walker SM. Weigle WO. Bacterial lipopolysaccharide (endotoxin) interferes with the induction of tolerance and primes thymus-derived lymphocytes. J Immunol. 126(3):938-42, 1981.