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Detrimental effects of cement mortar and fly ash mortar on asthma progression

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ABSTRACT

Currently, concrete additive materials are used worldwide to improve properties of concrete production and to reduce the total cost of the materials used in the concrete. However, the effects of exposure to various gases emitted from mortar mixed with additive materials are poorly understood. To evaluate the pattern of gas emission from cement mortar and additives, the emission levels of gas including ammonia (NH₃) and volatile organic compounds (VOCs) were measured from two different mortar types, Ordinary Portland Cement (OPC), and OPC with fly ash on various time points after manufacture. On days 1, 3, 10 and 30 after manufacture, moderate concentrations of NH₃ (4, 9, 12 and 5 ppm) were measured in OPC mortar (24 h, 150 mm × 150 mm × 50 mm), whereas higher concentrations of NH₃ (73, 55, 20 and 5 ppm) were measured in OPC mortar with fly ash (24 h, 150 mm × 150 mm × 50 mm). Furthermore, the concentration of VOCs was more than 10 ppm on 1, 3, and 10 days of age in OPC and OPC with fly ash mortars. To examine the mortars' allergic effects on the respiratory system, mice were sensitized with ovalbumin (OVA) and divided into four groups: normal, asthma control, OPC mortar and OPC mortar with fly ash. The mice were housed in corresponding group cage for 10 days with OVA challenges to induce asthma. Histopathologically, increased infiltration of lymphocytes was observed in the lung perivascular area of mice housed in OPC mortar and OPC mortar with fly ash cages compared to lungs of asthma control mice. Moreover, severe bronchial lumen obstruction and increased hypertrophy of bronchial epithelial cells ($p < 0.05$) were observed in the OPC mortar with fly ash group compared to OPC mortar or asthma control groups. Lungs of the two mortar groups generally expressed higher levels of genes related with asthma, including IL-4, eotaxin and epidermal growth factor (EGF) compared to lungs of asthma control mice. Additionally, the OPC mortar with fly ash group showed higher expression of IL-5, 13 and monocyte chemoattractant protein-1 (MCP-1) compared to the asthma control group. These results indicate that OPC mortar and OPC mortar with fly ash might exacerbate asthma.

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1. Introduction

In recent years, a variety of chemicals have been widely used as additives in concrete construction of buildings to improve properties of concrete production and to reduce the total cost of the materials used in the concrete. For instance, different types of waste materials including construction blast furnace slag (Cerulli et al., 2003), silica fume (GA, 2003) and fly ash (Li, 2005) were successfully tested for the preparation of dry mortars. Among the waste materials, fly ash is comprised of fine particles that include substantial amounts of silicon dioxide (SiO_2) and calcium oxide (CaO) from coal combustion. If cement can be manufactured with fly ash instead of clinker as its counterproposal, there would be many advantages, including the maximum use of industrial byproducts for high value-added resources, conservation of natural resources and reduction of production cost. While the amount and types of gas released depends on the composition of concrete materials, gases from the hydration process of cement concrete (mortar) can be released through fast diffusion in concrete and have a significant influence on public health via reductions in air pollution. The diffusion of these gases is becoming a serious problem throughout the world. In particular, ammonia gas (NH_3) is easily released in the high pH environment of concrete (Liang, 2001) and is thought to be harmful to human health when inhaled. Several researchers have reported that exposure of NH_3 above 1 ppm in a household can cause irritation of eyes, nose and throat (WHO, 1989). Exposure above 25 ppm of NH_3 can induce nausea, headaches and intense burning of eyes, nose, throat and skin. Sisovic and Kalinic (1987) investigated the relationship between indoor and outdoor NH_3 concentration in selected office buildings and found that the NH_3 concentration of indoor air exceeded several times that of outdoor air. Kobayashi (2000) reported that NH_3 generated from aggregates and cement concrete causes deterioration of linseed oil in oil paintings in newly constructed art museums. These results suggest that indoor NH_3 from concrete structures can cause serious health problems in humans.

Another substance similar to NH_3 that is emitted from concrete building materials is volatile organic compounds (VOCs). VOCs include numerous chemical compounds such as benzene, methylene chloride, perchloroethylene and formaldehyde. VOCs have been considered as pollutants that deteriorate indoor air quality in terms of odor annoyance, and induce eye and airway irritation, which leads to sick building syndrome (Weschler et al., 2006; WHO, 1989; Wolkoff et al., 2006; Woloff, 2001; Wolokff, 2001).

Despite of the harmful effects of NH_3 and VOCs on human health, the mechanism associated with allergic respiratory disease is unclear. Allergic diseases are caused by a combination of genetic and environmental factors, and recently, environmental factors have been considered as an important inducer of such disease progression. Asthma is a major allergic respiratory disease and is a complex syndrome of the airways characterized by variable and recurring symptoms in response to a variety of stimuli such as allergen inhalation, exposure to environmental pollutants or microbial infection (Anto, 2012; Cohen and Evans, 1992; Richardson et al., 2005).

Herein, we evaluated whether exposure to gases from pure cement mortar or cement mortar with fly ash affects the progression of respiratory disease. A mouse asthma model induced by ovalbumin (OVA) sensitization was used in the present study. We investigated whether exposure to gases from cement mortar with fly ash affects OVA-induced respiratory disease. Therefore, our findings may provide a basis for regulation of the use of unlimited and hazard cement supplements.

2. Materials and methods

2.1. Chemical compositions and mixture proportion of cement mortar

Ordinary Portland Cement (OPC) as specified in KS L 5201 (Portland cement, 2006) was used in the manufacture of mortar and the chemical compositions are shown in Table 1. Fly ash used was obtained from a T thermal power plant in Korea and had a Blaine specific surface area of approximately $3600 \text{ cm}^2/\text{g}$, and specific gravity of 2.1 g/cm^3 . Mortar was made at a 2.45:1 sand-to-cement ratio according to KS L 5105 (testing method for compressive strength of hydraulic cement mortar, 2007), and with a water-binder ratio (W/B) of 0.485. The size of mortar for analyzing the emission of NH_3 was $150 \text{ mm} \times 150 \text{ mm} \times 50 \text{ mm}$ and the mortar was placed into the environmental chamber. The environmental chamber was then immediately sealed and cured for 24 h at a constant humidity and temperature of $20 \pm 2^\circ\text{C}$. The load factors of samples used in the environmental chamber were $1.0 \text{ m}^2/\text{m}^3$ ($\pm 10\%$). Gas sampling was performed by collecting the gases directly in a Teflon pocket using an MFC pump for gases released from cement mortar within the early days of the hydration stage. As indicated in Table 2, the sample of OPC mortar with fly ash was prepared by a 1:1 mixture ratio of fly ash to OPC. A cement mortar cage was fabricated according to the same protocol (Tables 1 and 2) and the size of the mortar cage for housing the mice was $230 \text{ mm} \times 170 \text{ mm} \times 130 \text{ mm}$.

2.2. Measurement of gas

The concentration of NH_3 was measured in accordance with KS I 2218 (detector tube type gas measuring instruments, 2009). A cylinder-style gas detector employing the vacuum method was used and the gas was absorbed in the detecting tube by decompressing the inside of the cylinder after connecting a small Teflon pocket to the detecting tube (Fig. 1).

2.3. Animals and experimental protocol

Six-week-old female BALB/c mice were purchased from Taconic Farms, Inc. (Samtako Bio Korea, O-San, Korea). All animals were maintained at $22\text{--}24^\circ\text{C}$ and a relative humidity of 50–70% with a 12 h light/dark cycle. Food and water were supplied *ad libitum*. Animal experiments were performed according to the guidelines of the Institutional Animal Care and Use Committee of Chonbuk National University.

Mice were sensitized on days 0, 7 and 14 by intraperitoneal injection of $20 \mu\text{g}$ of OVA (Sigma-Aldrich, St. Louis,

Table 1 – Chemical composition of Ordinary Portland Cement and fly ash.

	SiO ₂	CaO	Al ₂ O ₃	Fe ₂ O ₃	Na ₂ O	K ₂ O	SO ₃	MgO	TiO ₂	LOI
OPC	20.88	64.73	5.39	2.38	0.27	0.22	1.65	1.51	1.33	2.04
Fly ash	54.96	5.18	23.02	7.35	0.33	0.86	4.58	1.39	1.23	4.16

Table 2 – Mix proportions of the mortar.

Type	Binder		Sand (g)	Water-binder ratio (%)
	OPC (g)	Fly ash (g)		
OPC	1000	–	2450	48.5
OPC + fly ash	500	500	2450	48.5

MO, USA) adsorbed to 1 mg of aluminum hydroxide (Alum) (Sigma–Aldrich) in 200 μ l of sterile saline (Fig. 2). Non-sensitized normal mice received 1 mg of Alum. OVA-sensitized mice were placed in a plastic chamber and then exposed to an aerosol of OVA (10 mg/ml in 0.9% saline) for 20 min at 21, 24, 27 and 30 days after the first intraperitoneal OVA injection.

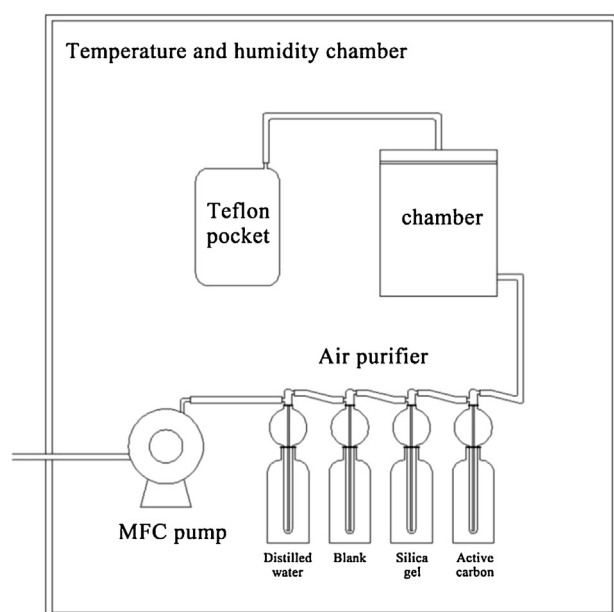
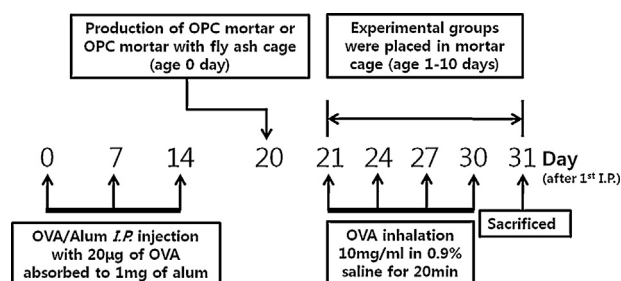
**Fig. 1 – Schematic diagram of gas sampling systems.**

Fig. 2 – Challenge protocol of OVA-induced asthma model. Each group contained 6–7 mice. Mice were immunized to OVA by intraperitoneal injection of 20 μ g of OVA adsorbed to Alum on 0, 7 and 14 days after the first OVA/Alum injection. From day 21 to day 30, mice were exposed to an aerosol of OVA (10 mg/ml) in 0.9% saline for 20 min using an ultrasonic nebulizer. On day 31 after the first injection, mice were sacrificed and the pathologic factors evaluated.

Mice were divided into the following four groups: normal (mock control, $n=6$), asthma control ($n=6$), OPC mortar ($n=7$) and OPC mortar with fly ash ($n=7$) (Table 3). At first, all mice were housed in common plastic cages. On 21 days after the first intraperitoneal OVA injection, OPC mortar and OPC mortar with fly ash groups were each housed in a same-sized cement mortar cage (230 mm $\times$ 170 mm $\times$ 130 mm) manufactured after a 24 h hold, while other groups remained in the original plastic cages. From 21 to 31 days after the first intraperitoneal OVA injection, all experimental cages were covered by 0.3 μ m HEPA filter to observe the effect of cement mortar cages. On 31 days after the first intraperitoneal OVA injection, mice were sacrificed and lungs and serum sample were collected. The right lung lobes were stored at -70°C until used.

2.4. Histopathological evaluation

For histopathological observation of lung sections, the left lung lobes were preserved in 10% buffered formalin and routinely processed, embedded in paraffin, sectioned at 4 μ m thickness, stained with hematoxylin and eosin (H&E), and examined with light microscopy. Total lung section images were analyzed by light microscopy (BX-51, Olympus Corp., Tokyo, Japan) with the analySIS TS Auto software (Olympus Corp., Tokyo, Japan).

2.5. Measurement of serum cortisol and IgE

Blood samples were collected from mice by direct heart puncture, and centrifuged at 2000 rpm for 10 min. The sera were then carefully collected and stored at -80°C until further use. For the detection of serum cortisol, radioimmunoassay (RIA, coated tube method) was performed using a COAT-A-COUNT Cortisol kit (Seimens, USA). Immunoglobulin E (IgE) was measured in serum by cytokine-specific sandwich ELISA. Briefly, ELISA plates (Greiner Bio-One, Germany) were coated with purified anti-mouse IgE antibody (1:500) (BD OptEIA™, San Diego, CA, USA) and incubated at 4°C overnight. The plates were washed with 0.05% Tween 20/PBS and blocked with 10% FBS/PBS for 1 h at room temperature. After washing, the serial two fold diluted samples and standards were added to wells and incubated overnight at 4°C . After washing, biotin anti-mouse IgE antibody (1:250) (BD OptEIA™, San Diego, CA, USA) was added to the wells and incubated at room temperature for 1 h. Avidin horseradish peroxidase (eBiosciences, San Diego, CA, USA) was added to the plates for cytokine detection. Finally, a 3,3',5,5'-tetramethylbenzidine (TMB) substrate

Table 3 – Experimental groups and treatments.

Condition	Environment	Group
Normal	Normal plastic cage	Normal Asthma control
OVA induced asthma	Cement mortar cage	Ordinary Portland Cement (OPC) Mortar OPC Mortar with fly ash

reagent set (BD Biosciences, San Diego, CA, USA) was used for color development, and the reaction was stopped with 2 M H_2SO_4 . Plate reading was conducted at 450 nm using an ELISA reader.

2.6. Quantitative real-time PCR

Total RNA was extracted from lungs using an Easy-Spin Total RNA extraction kit (iNtRon Biotech, Seoul, Korea), and both the nucleic acid concentration and purity were measured with a NanoDrop® ND-1000 (NanoDrop Technologies, Wilmington, DE, USA). Following incubation with RNase-free DNase I (Promega, Madison, WI, USA), reverse transcription was performed using a PrimeScript™ RT reagent kit (Takara Bio Inc., Shiga, Japan), following the manufacturer's instructions. The resulting cDNA was subjected to real-time PCR on a CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) using SYBR Green II as a double-strand DNA-specific binding dye to quantify the expressions of IL-4, IL-5, IL-10, IL-13, eotaxin, monocyte chemoattractant protein-1 (MCP-1), epidermal growth factor (EGF), and intercellular adhesion molecule-1 (ICAM-1) in lungs. PCR amplifications were performed with specific primers in a total volume of 20 μl containing 5 pmol of each primer, 10 μl 2 $\times$ SYBR® Premix Ex Taq™ II (Takara Bio Inc., Japan), and nuclease-free PCR-grade water after an initial denaturation at 95 °C for 10 s and 45 cycles (95 °C for 5 s and 61 °C for 30 s). After completion of the reaction, specificity was verified by performing a melting curve analysis. Quantification was performed by comparing the Ct values of each sample with normalization to GAPDH.

2.7. Statistical analysis

All data were expressed as mean $\pm$ standard error. Differences between groups were compared using a two-tailed Student's t-test with SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA). A *p*-value of <0.05 was considered significantly.

3. Results

3.1. Measurement of gases emitted from OPC mortar and OPC mortar with fly ash

To investigate the concentration of NH_3 released from OPC and OPC with fly ash mortars (150 mm $\times$ 150 mm $\times$ 50 mm), the gas was measured in the environmental chamber using an NH_3 detecting tube at different time points after manufacture. Although moderate amounts of NH_3 (4 ppm) were measured in OPC at 1 day post-manufacture, the peak concentration of NH_3 (12 ppm) released from OPC was at the age of 10 days and the concentration of NH_3 declined but was still detectable at

day 30 (Fig. 3). Compared with the OPC mortar cage, a concentration of NH_3 (73 ppm) almost 18 times higher was measured in the OPC mortar with fly ash cage at day 1 after manufacture compared to OPC only. The emission of NH_3 from OPC mortar with fly ash gradually declined until day 30 post-manufacture, but was still higher than OPC mortar only at day 3 and 10 post-preparation. These results indicate that the patterns of NH_3 release are different between OPC mortar only and OPC mortar with fly ash, and considerable amount of NH_3 is emitted from OPC mortar with fly ash.

In addition to NH_3 , concentration of VOCs released from OPC mortar and OPC mortar with fly ash was measured using a polytec detecting tube (data not shown). Different from the NH_3 emission pattern, within the VOCs species, the concentration of toluene and xylene was more than 10 ppm at 1, 3, and 10 days of age and less than 10 ppm at 30 days of age in OPC and OPC with fly ash mortars although the exact quantitative measurement of VOCs was not performed because a polytec detecting tube do not provide a quantitative measure.

3.2. Effects of cement exposure on OVA-induced asthma

To determine whether exposure to different housing components affects the asthma-like respiratory symptoms, mice presensitized with OVA were housed in different cages (plastic, OPC mortar, OPC mortar with fly ash) for 10 days. The OVA challenge was performed serially four times during this period and mice were analyzed for severity of asthmatic symptoms. OVA-induced asthma mice were characterized by pathological changes in lungs, such as abnormal bronchial epithelial cell hypertrophy, exudates secretion in the airways, and

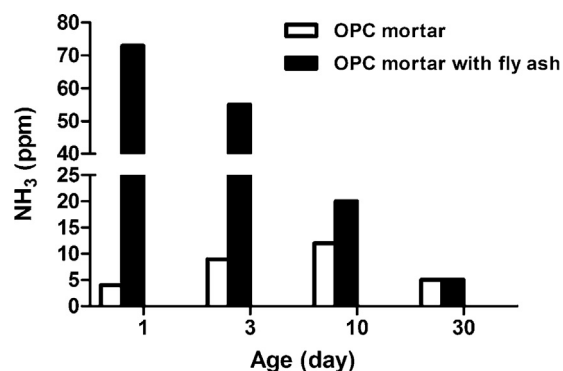


Fig. 3 – Time-dependent NH_3 emissions in OPC mortar and OPC mortar with fly ash after manufacture. At 24 h, NH_3 gases emitted from OPC mortar and OPC mortar with fly ash (150 mm $\times$ 150 mm $\times$ 50 mm) were measured by a NH_3 detecting tube in the environmental chamber (1, 3, 10, and 30 days of age).

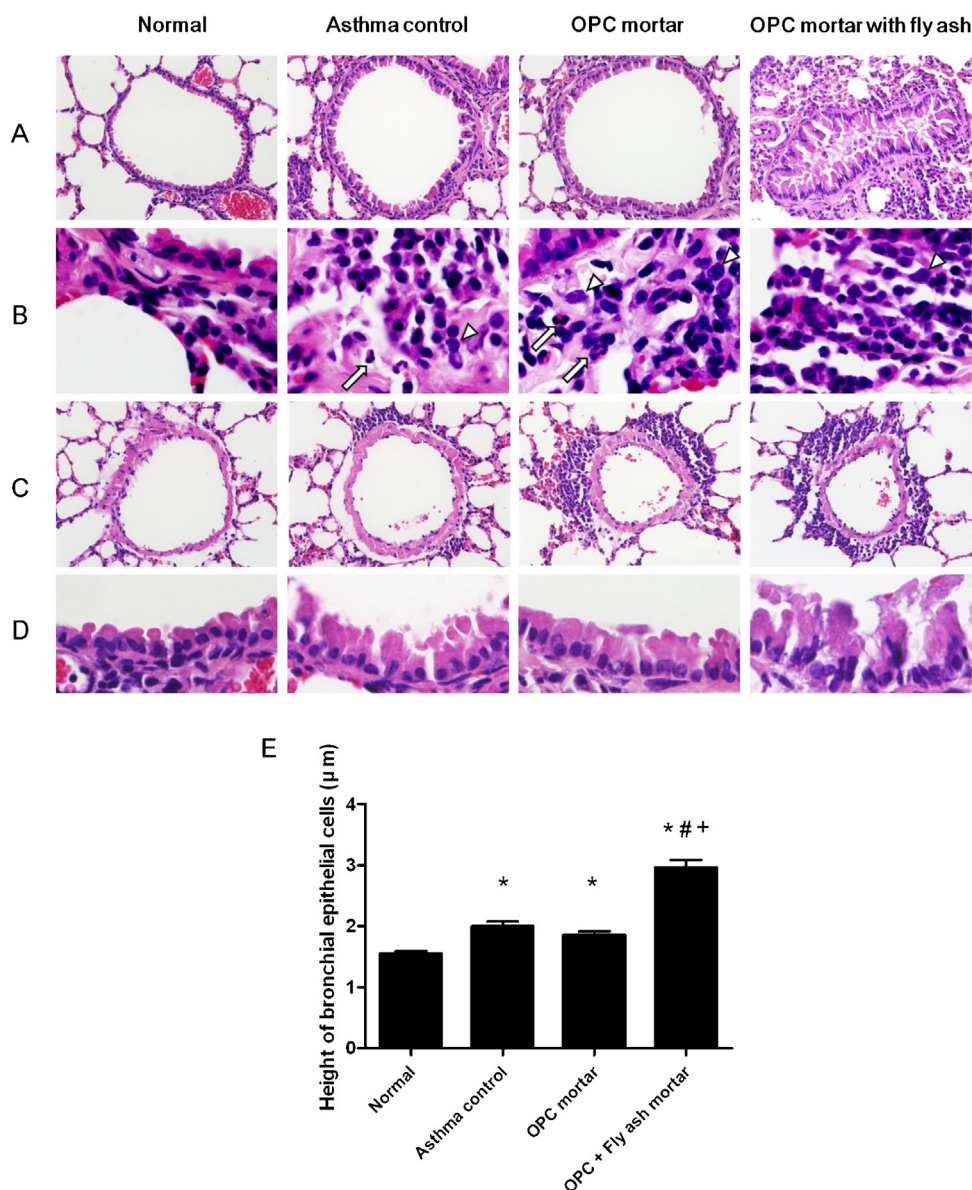


Fig. 4 – OPC mortar with fly ash group increased histopathologic lesions of asthma. Histological features of the lung sections ($n = 6-7$ lung per each group) from the experimental group 31 days after the first injection: (A) bronchi/bronchiole (×400); (B) inflammatory cells in the peribronchial area (×1000). Eosinophil (arrow), mast cells (arrow head) and lymphocytes were observed; (C) blood vessel (×400); (D) bronchial epithelial cell (magnified figure of A). Sections were stained with hematoxylin and eosin. (E) The average height of bronchial epithelial cell (μm) by imaging analysis program. * $p < 0.05$ when compared to normal; # $p < 0.05$ when compared to asthma control; + $p < 0.05$ when compared to OPC mortar. Values represent mean $\pm$ SEM.

inflammatory cell infiltration in perivascular-parenchymal areas (Cohen and Evans, 1992; Huerta-Yepez et al., 2008). As shown in Fig. 4, the lungs of asthma control and OPC mortar group mice showed similar degrees of bronchial epithelial cell hypertrophy and infiltration of inflammatory cells including eosinophils, lymphocytes and mast cells in the peribronchial area. Furthermore, the average height of bronchial epithelial cells in the OPC mortar group was similar to the asthma control group (Fig. 4E). However, increased infiltration of lymphocytes was observed in the lung perivascular area in the OPC mortar group compared to asthma control group (Fig. 4C). Interestingly, more severe bronchial lumen obstruction with

inflammatory cells, necrotic debris and mucous in the OPC mortar with fly ash group was observed compared to OPC mortar and asthma control groups (Fig. 4A). Furthermore, significantly increased hypertrophy of bronchial epithelial cells was measured in the OPC mortar with fly ash group compared to OPC mortar and asthma control groups (Fig. 4D and E). In the perivascular area, the degree of lymphocyte infiltration increased similarly to the OPC mortar group (Fig. 4C). The results indicate that respiratory exposure to OPC mortar only or to OPC mortar with fly ash, deteriorates asthma-related lung pathology with OPC mortar with fly ash being the strongest inducer of asthma progression.

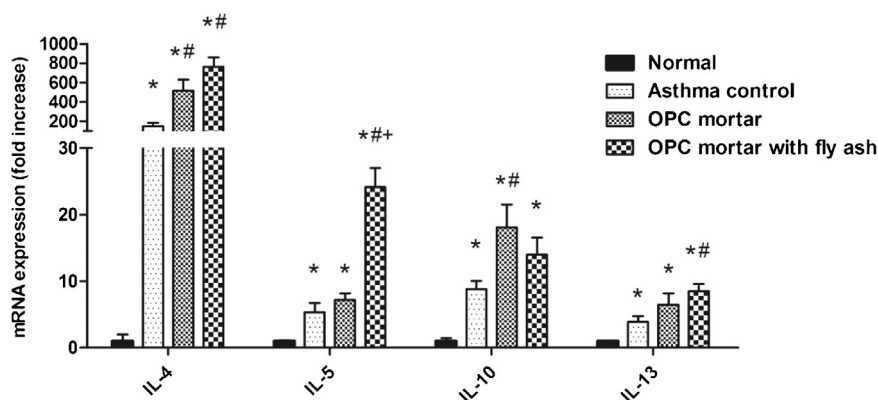


Fig. 5 – OPC mortar and OPC mortar with fly ash group showed increased mRNA expression of Th2 type cytokines in the lung. The mRNA expression of IL-4, IL-5, IL-10 and IL-13 was analyzed from normal ($n = 6$), asthma control ($n = 6$), OPC mortar ($n = 7$) and OPC mortar with fly ash groups ($n = 7$) by real-time PCR. * $p < 0.05$ when compared to normal; # $p < 0.05$ when compared to asthma control; + $p < 0.05$ when compared to OPC mortar. Values represent mean $\pm$ SEM.

3.3. Evaluation of the expression of asthma-related genes in the lung

Overwhelming evidence suggest that Th2 type cytokines such as IL-4, -5, -10 and -13, and chemokines such as eotaxin and MCP-1 produced by eosinophils, mast cells and Th2 type T lymphocytes are primary mediators for inducing asthma (Hamid, 2003; Kim et al., 2010; Walsh, 2010). Therefore, high level expression of the aforementioned cytokines is well known to be positively correlated with clinical asthma severity and progression. To determine the effects of cement mortar or fly ash on asthma-related gene expression, mRNA expression of IL-4, -5, -10, and -13 in the lungs from individual mice housed in different cages was measured. As shown in Fig. 5, the lungs of mice housed in OPC mortar with fly ash cages generally expressed higher levels of IL-4, -5, and -13, except IL-10, compared to lungs of the asthma control mice. The lungs of mice housed in OPC mortar cages expressed higher levels of IL-4 and IL-10 than the lungs of asthma control mice, while no significant differences of IL-5 and 13 expression were observed between asthma control and OPC mortar groups. These results suggest that OPC mortar only, OPC mortar with fly ash, or both induce asthma-related gene expression in the lung sensitized with OVA, which supports previous data (Fig. 4) that exposure to OPC mortar only or OPC mortar with fly ash increases asthma severity.

Eotaxin is known as a potent eosinophil chemoattractant (Paplinska et al., 2007; Pease and Williams, 2001) and MCP-1 is also known as a chemoattractant of monocytes to sites of tissue injury, infection, and inflammation (Szalai et al., 2001). Asthma patients show elevated eotaxin and MCP-1 levels in serum, indicating that elevation of MCP-1 and eotaxin might be used to predict the onset of asthma (Jahnz-Rozyk et al., 1997; Lilly et al., 1999). As expected, all OVA-sensitized groups revealed higher mRNA expression of eotaxin and MCP-1 compared to the mock control group (Fig. 6A). In the OPC mortar and OPC mortar with fly ash groups, levels of eotaxin in the lungs were significantly increased compared to asthma control mice. Only the OPC mortar with fly ash group showed a significant increase of MCP-1 expression compared to other

groups, indicating that asthma-related inflammation was most severe in the lungs of mice housed in OPC mortar with fly ash cages. In addition to Th2 type cytokines and chemokines, EGF is also a predictor gene for asthma progression since EGF is involved in stimulating angiogenesis and airway smooth muscle proliferation (Le Cras et al., 2011; Tsuchiya et al., 2010). EGF expression in the lungs of mice housed in both OPC mortar and OPC mortar with fly ash cages was higher than in the lungs of the asthma control mice (Fig. 6B). The function of ICAM-1 on asthma is controversial, however a positive relation of ICAM-1 expression and asthma progression is generally accepted (Stanciu and Djukanovic, 1998; Tang and Fiscus, 2001). In the lungs of the mice in the OPC mortar group, there was no significant difference of ICAM-1 expression compared to mock control and asthma control groups. However, a significantly decreased expression of ICAM-1 was measured in the lungs of mice in the OPC mortar with fly ash group compared to mock control and asthma control groups (Fig. 6B). As shown in Figs. 5 and 6A, the lungs of mice in the OPC and OPC with fly ash groups expressed higher levels of genes related with Th2 type immune response compared to asthma control mice. Furthermore, the OPC mortar with fly ash group showed more severe asthma progression compared to the OPC mortar group.

3.4. Serum cortisol and IgE levels

Because cortisol level is known to decrease in patients with severe asthma (Eid et al., 2002; Landstra et al., 2002), but increase in stressful conditions (Goyal et al., 2011), we examined whether a cement cage environment affects serum cortisol levels in mice. Serum cortisol levels were decreased in the mice sensitized with OVA and housed in a plastic cage compared to the mock control group (Fig. 7A). Interestingly, serum cortisol levels increased in mice housed in an OPC mortar cage although a statistical difference was not found between the groups. In contrast, serum cortisol levels were not increased in mice housed in OPC mortar with fly ash cages compared to the asthma control group.

An increase of IgE concentration is commonly associated with allergy and asthma in humans and animals (Tanizaki

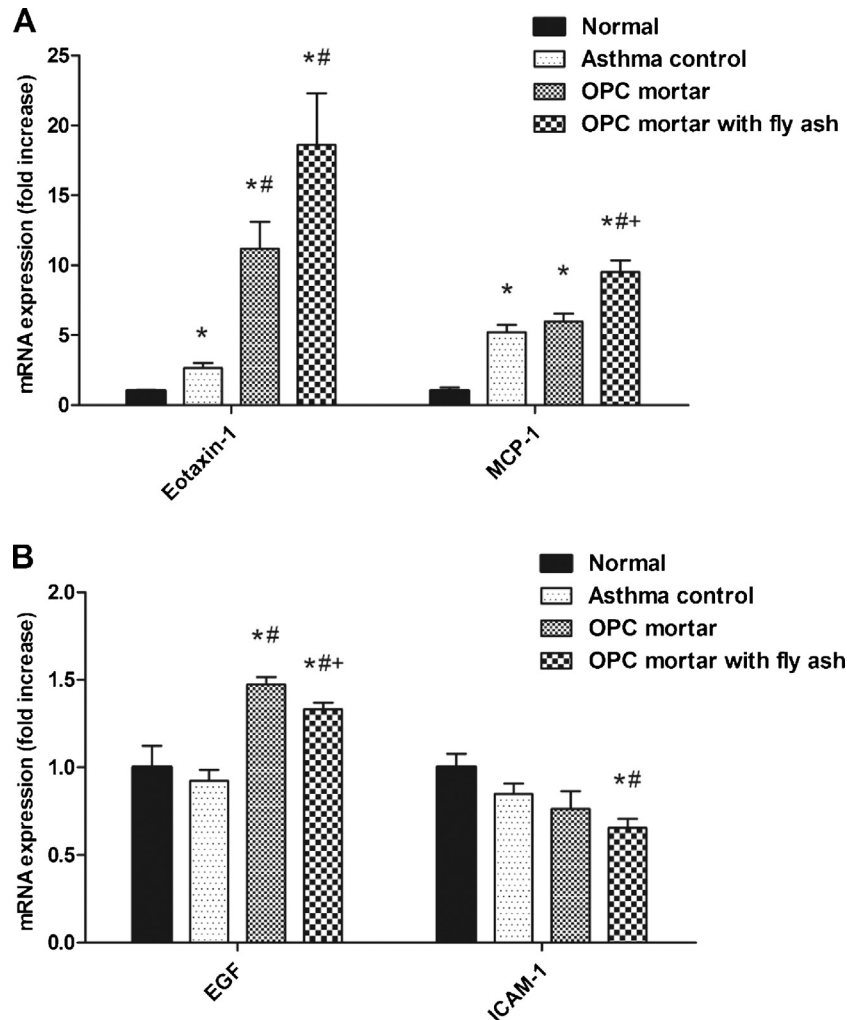


Fig. 6 – Changes of asthma-related gene expression by OPC mortar and OPC with fly ash in the lung. (A) Eotaxin and MCP-1 mRNA expression, (B) EGF and ICAM-1 mRNA expression. The mRNA expression was analyzed from normal ($n = 6$), asthma control ($n = 6$), OPC mortar ($n = 7$) and OPC mortar with fly ash groups ($n = 7$) by real-time PCR. * $p < 0.05$ when compared to normal; # $p < 0.05$ when compared to asthma control; + $p < 0.05$ when compared to OPC mortar. Values represent mean $\pm$ SEM.

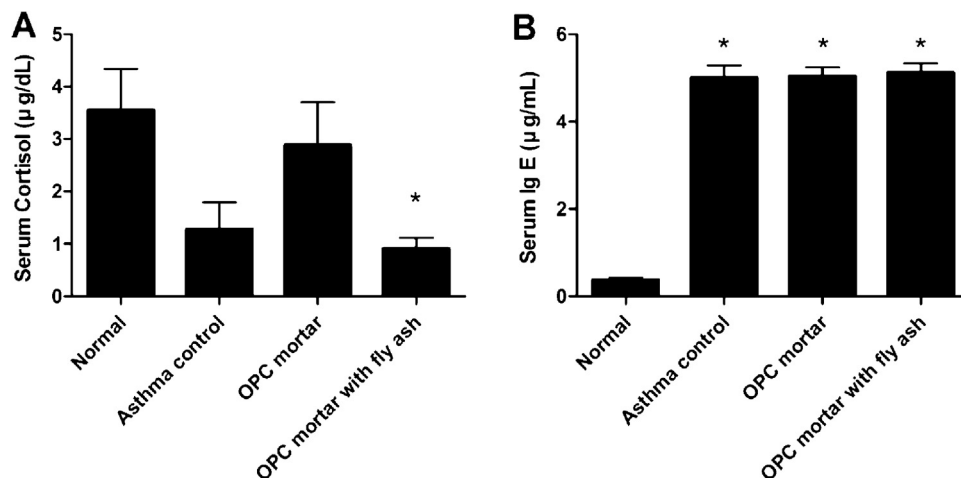


Fig. 7 – Comparison of serum cortisol and serum IgE levels between asthma control and mortar groups (OPC mortar, OPC mortar with fly ash). (A) Serum cortisol ($\mu\text{g/dL}$) determined by RIA. (B) Serum IgE ($\mu\text{g/mL}$) determined by ELISA. * $p < 0.05$ when compared to normal. Values represent mean $\pm$ SEM.

et al., 1992). To determine the effects of cement mortar or fly ash on IgE-mediated asthma progression, serum IgE levels were measured by ELISA. Serum IgE levels were greater in OVA-induced asthma mice compared with normal mice ($p < 0.05$) (Fig. 7B). However, IgE levels did not differ between the mice groups housed in plastic cages (asthma control) and cement mortar cages (OPC, OPC with fly ash).

4. Discussion

Based on studies regarding the relationship between environmental factors and asthma disease, allergens and irritating chemicals from environment have been shown to aggravate asthma symptoms in humans (Anto, 2012; Cohen and Evans, 1992; King et al., 2004; Krieger et al., 2010; Richardson et al., 2005). Moreover, for cost-saving purposes, extenders such as fly ash are used in many construction sites worldwide and this practice is rapidly growing (GA, 2003; KISC, 2010). In spite of harmful effects on human health, how the extenders can induce disease and what types of chemicals are associated with respiratory problems is unclear. Therefore, we analyzed the emission pattern of NH_3 and VOCs from mortar and mortar with fly ash at different time points after manufacture, and investigated their effects on the respiratory system.

According to the World Health Organization report, exposure above 1 ppm NH_3 in a household can cause irritation of the eyes, nose and throat, and exposure of 25 ppm NH_3 can cause serious burns and permanent damage to the eyes, lungs and skin (WHO, 1989). The US Occupational Safety and Health Administration has set a short-term (15 min) exposure limit of NH_3 as 35 ppm (OSHA, 2004). Furthermore, the National Institute for Occupational Safety and Health recommends that the level of NH_3 in workroom air should be limited to 50 ppm for 5 min (NIOSH, 1992). In the present study, the ongoing emission of NH_3 was found from both OPC mortar and OPC mortar with fly ash (150 mm $\times$ 150 mm $\times$ 50 mm) during 30 days after preparation (Fig. 3). Moreover, the mortar including fly ash material showed a significantly increased NH_3 concentration compared with OPC mortar. Accordingly, these results indicate that fly ash is responsible for the major source of NH_3 emission and this type of environment may deteriorate asthma-related respiratory symptoms.

VOCs including carcinogens such as toluene and benzene are emitted during the construction of a cement structure (Ware et al., 1993). VOCs reportedly have been implicated as causative agents in asthma and building-related illness (Pappas et al., 2000). In the present study, the concentration of VOCs was measured in cement mortar cages with and without fly ash at 1, 3, and 10 days of age and the concentration of toluene and xylene was more than 10 ppm (data not shown). However, because of technical limitations, the quantitative analysis of all types of VOCs from both OPC mortar and OPC mortar with fly ash at different time points could not be performed. Therefore, VOCs emission may also be related with the progress of respiratory diseases, but whether the major source of VOCs emission is OPC mortar or OPC mortar with fly ash is unclear in the present study.

In our preliminary experiment to evaluate toxicological effects of OPC mortar in normal mice, OPC mortar and OPC

mortar with fly ash did not show any respiratory pathologic changes compared to control group (data not shown). Therefore, we concluded that respiratory stimuli from OPC mortar and OPC mortar with fly ash were not strong enough to cause a pathological problem in healthy humans or animals. However, unlike healthy people, severe symptoms caused by environmental chemicals can occur in patients with asthma diseases. Asthma diseases are thought to be due to a combination of genetic and environmental factors, and the latter are generally considered as important inducers for respiratory disease progression (Richardson et al., 2005). Therefore, our study focused on asthma disease to determine the effects of OPC mortar and OPC mortar with fly ash on the allergic diseases using an OVA-induced asthma model.

Perivascular infiltration of lymphocytes is a type of characterized asthma lesion and vascular endothelial cells are one factor related to lymphocyte emigration (Kuebler et al., 1997). After endothelial cell damage, migrated T cells participate in pulmonary vascular remodeling and this immune response may contribute to the pathogenesis of inflammatory vascular lesions (Cuttica et al., 2011). According to the histopathologic data from the present study, the mice housed in OPC mortar with fly ash cages showed accelerated asthma progression compared to asthma control and OPC mortar groups (Fig. 4). Interestingly, similar to the OPC mortar with fly ash group, increased perivascular infiltration of lymphocytes was observed in the OPC mortar group compared to the asthma control group. Based on these results, OPC mortar and OPC mortar with fly ash was hypothesized to cause vascular endothelial cell damage in the lung tissue of asthma mice.

Because ICAM-1 is implicated in cell adhesion, we investigated the potential modulation of ICAM-1 expression in lung tissues exposed to OPC mortar and OPC mortar with fly ash. Although, increased ICAM-1 expression in asthma is generally related with lymphocyte infiltration and asthma progression, our data (Fig. 6B) showed significantly decreased expression of ICAM-1 in the lungs of mice in the OPC mortar with fly ash group compared to mock control and asthma control groups indicating the decreased ICAM-1 expression observed was possibly due to an unknown mechanism. Just et al. (2003) reported that keratinocyte growth factor (KGF) decreases ICAM-1 expression in bronchial epithelial cells. KGF is an epithelial cell-specific growth factor secreted by fibroblasts and appears to have an important role in pulmonary as well as bronchial epithelial repair after inflammation and injury. Release of proinflammatory cytokines leads to an increase of KGF production (Brauchle et al., 1994; Werner, 1998). However, the detailed mechanism of decreased ICAM-1 expression by fibroblast growth factors such as KGF in lungs is not clearly understood. Therefore, the present study's result regarding ICAM-1 is assumed to be associated with changes of the expression of fibroblast growth factors and proinflammatory cytokines.

Cortisol has been known as a stress hormone and has immunosuppressive function (Anagnostis et al., 2009). Based on data shown in Fig. 7A, different functions of cortisol may be involved in the different experimental groups. The histopathology and RT-PCR data suggest that mice housed in OPC mortar or OPC mortar with fly ash cages expressed moderately or severely higher severity of asthma compared to mice

housed in plastic cages. A higher level of serum cortisol in mice housed in OPC mortar cages was thought to be caused by higher stress induced by exposure to NH_3 and VOCs. However, cortisol levels of the OPC mortar with fly ash group returned to the level of the asthma control group, indicating that severe asthma progression rather than stress induced by the cement cage was the main cause of suppressed cortisol production, but further detailed studies are needed.

Increased serum IgE indicates the onset of an asthma-like state in the OVA model (Tanizaki et al., 1992). However, as shown in Fig. 7B, no differences were observed between mice groups housed in plastic cages (asthma control) and cement mortar cages (OPC mortar and OPC mortar with fly ash). Reportedly, serum IgE measurement is limited when evaluating asthma severity because of restricted production in the initial stage of asthma. Late-stage asthma is characterized by the presence of cytokines, chemokines, growth factors and proteases (Peden, 2002; Van Hove et al., 2008). Therefore, the facts suggest that OPC mortar and OPC mortar with fly ash do not affect OVA-related induction of asthma but exacerbate the inflammatory environment as demonstrated by histopathology and RT-PCR results in the present study, thus possibly explaining why serum IgE levels were similar between the groups although asthma severity was different.

The toxicity of OPC mortar and OPC mortar with fly ash in an OVA-induced asthma mice model, which have more sensitivity in respiratory toxicology, was evaluated in the present study. The OPC mortar group did not show greatly increased asthmatic factors compared with the asthma control group. Conversely, the OPC mortar containing fly ash significantly presented severe asthmatic factors among the groups, indicating OPC mortar with fly ash, rather than OPC mortar only, has more potential toxicity on the respiratory system and can be an exacerbating factor in asthma progression. Therefore, further detailed studies are necessary to explain the mechanistic effects of gases emitted from cement mortar and concrete additive materials on respiratory symptoms such as asthma.

5. Conclusion

The exposure of OPC mortar or OPC mortar with fly ash on mice having asthma clearly showed that OPC mortar with fly ash induced more detrimental respiratory symptom effects than the OPC mortar. The underlying mechanisms of asthma progression caused by OPC mortar with fly ash may involve increased Th2 type immune responses. The negative effects may be attributed to emission of NH_3 as well as VOCs from mortar with fly ash. Based on the results from the present study, OPC mortar only or OPC mortar with fly ash may be a negative factor exacerbating asthma. Therefore, further studies are required to clarify whether various concrete additives influence the progression of respiratory symptoms.

Conflict of interest statement

Nothing declared.

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