

A Murine Model of Acute Allergic Conjunctivitis Induced by Continuous Exposure to Particulate Matter 2.5

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PURPOSE. Several pieces of epidemiologic evidence have indicated PM2.5 (particulate matter with a diameter of 2.5 μm or less) as a causing factor of allergic conjunctivitis, but without experimental elucidation of mechanism. In the present study, PM2.5 in solution was directly applied to the mouse ocular surface to elucidate whether PM2.5 might cause allergic conjunctivitis, and its underlying mechanisms were analyzed.

METHODS. ICR mice were challenged for 18 consecutive days with eye drops containing PM2.5 at 3.2, 6.4, and 12.8 mg/mL in 0.9% NaCl saline, along with the controls prepared in parallel without PM2.5 and another control group treated with both PM2.5 at 12.8 mg/mL and artificial tears. On day 19, the whole eyes and meibomian glands were harvested for histopathological analyses and assessment of clinical scoring, tear volume, tear breakup time, and tear ferning. Furthermore, goblet cells by periodic acid Schiff stain and infiltrated eosinophils by Giemsa stain were quantified and compared among study groups.

RESULTS. Clinical scoring showed more eyelid edema, tearing, and scratching behaviors, with longer tear breakup time under the influence of increased PM2.5 concentrations. Tear ferning assay showed less tear crystal formation and decreased crystal branches after exposure to PM2.5. In addition, higher goblet cell density in the upper palpebral conjunctiva and extensive eosinophil infiltration in the entire conjunctiva and in the meibomian glands were induced by PM2.5.

CONCLUSIONS. These results demonstrate that PM2.5 can induce symptoms similar to clinical allergic conjunctivitis and that the murine acute allergic conjunctivitis model can be induced by direct exposure to PM2.5.

Keywords: allergic conjunctivitis, PM2.5, mouse model

Particulate matter 2.5 (PM2.5), referring to particulate matter with diameter less than 2.5 μm , has a very complex composition, including, for example, pollen, dust, smoke, and industrial exhaust.¹ PM2.5 can be suspended in the air for long periods of time and is believed to pose risks to health.^{2–6}

Allergic conjunctivitis (AC) is an eye inflammation condition caused by allergic reactions to substances such as pollen or mild spores.⁷ It is one of the most common ocular surface diseases encountered in clinical practice and may come in either acute or chronic types.⁸ The major clinical signs of AC consist of chemosis, tearing, conjunctival hyperemia, and eyelid edema.^{9,10} Since the eye is exposed directly to the outside world, allergens brought by PM2.5 are readily in contact with the ocular surface¹¹ and may potentially induce AC.

To elucidate the effects of airborne particulate matter, including PM2.5, on the ocular surface, previous studies using cell lines^{12–15} and human clinical studies^{16,17} have been performed. The scenario induced by PM2.5 or related airborne particulates included autophagy,^{12,13} DNA damage, and cell

senescence in cornea epithelial cells,¹⁴ increased production of proinflammatory cytokines and mucin,¹⁵ and tear osmolarity changes.¹⁶

Previous animal studies addressing PM2.5-induced cornea damage are relatively few. One study reported by Cui et al.¹⁸ demonstrated that PM2.5 may delay cornea epithelium wound healing. Another report by Tan et al.¹⁹ showed that PM2.5 exposure led to dry eye syndrome. Although both these animal study reports did not refer to PM2.5 as a factor causing AC, several lines of evidence derived from epidemiologic studies^{20–22} have related PM2.5 exposure to AC symptoms. Hence, it is likely that exposure to PM2.5 may also lead to AC pathogenesis. However, to our knowledge, no experimental data addressing PM2.5-induced AC have been reported in an animal model.

The current agents used for AC treatments include antihistamines, mast cell stabilizers, nonsteroidal anti-inflammatory drugs, and topical corticosteroids.^{23,24} These agents are known to cause side effects after long-term use. As such,



further research is mandatory for development of better treatment regimens. Particularly, animal models that closely resemble human allergic AC must be developed. Since continuous exposure to PM2.5 has been reported to cause allergic inflammation in the lung²⁵ and in the nasal cavity,²⁶ we hypothesized that direct PM2.5 application to the ocular surface would induce AC symptoms. In the present study, we determined the protocol and the dose of PM2.5 that would be sufficient to induce AC in a mouse model, with detailed analyses of the characteristics that mimic the human conditions.

MATERIALS AND METHODS

PM2.5 Collection, Heavy Metal Contents Analysis, and Eye Drop Preparation

The collection was performed at Chung Shan Medical University, Taichung City, Taiwan, between October 1, 2017 and December 31, 2017. PM2.5 was collected with a high-volume sampler (model TE-6070V equipped with TE-231 High Volume Cascade Impactor; Tisch Environmental, Cleves, OH, USA), simultaneously using two quartz microfiber filters (PM2.5-10: 8" × 10"; PM2.5: 2.625" × 5.375") at a constant flow rate of 1.3 m³/min. Sampling was conducted continuously for 24 hours from 8:00 AM to 8:00 AM the next day. The samples were extracted only from the PM2.5 sampling filter strips. The strips were immersed in deionized water and sonicated with a water-bath sonicator for 30 minutes. The particulate matter suspensions were then dried in a vacuum frozen desiccator, weighed, and stored at 4°C. The heavy metal composition of the PM2.5 samples was analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (NexION300X; PerkinElmer, Akron, OH, USA). For preparation of PM2.5 eye drops, the samples were resuspended to 3.2, 6.4, and 12.8 mg/mL in 0.9% NaCl saline just before use. The three doses were determined according to the average concentrations regarded as normal, risky to sensitive populations, and risky to all populations in 24 hours.²⁷

Animals

Fifty 6- to 8-week-old female ICR mice (purchased from LASC0 Ltd., Taiwan) were housed in an animal facility maintained at 20° to 24°C with 50% to 60% humidity under a 12-hour light/12-hour dark cycle, and fed with a commercial diet and given water ad libitum. All animal care and treatment protocols were performed in accordance with the standard laboratory animal protocols approved by the Institutional Animal Care and Use Committee (Chung Shan Medical University, Taichung, Taiwan), and all experimental procedures were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Study Groups and PM2.5 Applications

Mice were randomly divided into five groups of 10 each. Group 1, blank control group, was treated with direct dropping of 0.9% NaCl saline on the ocular surface. Groups 2 to 4 were treated, respectively, with PM2.5 at 3.2, 6.4, and 12.8 mg/mL in 0.9% NaCl saline by dropping directly on the ocular surface; group 5, the remission group, was treated with direct dropping of PM2.5 at 12.8 mg/mL in 0.9% NaCl saline with artificial tears (AT) treatment. The AT (ALLERGAN, Refresh TEARS, Waco, TX, USA) were applied by 10 minutes after each eye had been exposed to PM2.5. All eye drops, including AT, were applied

approximately at 5 μL on each eye, three times a day at 9:00 AM, 1:00 PM, and 5:00 PM, during a 19-day experiment period.

Data Collection and Assessment Schedule

All clinical symptoms were video recorded and photographed for further analyses. The pretests were performed 1 day prior to the first day of PM2.5 treatment (day 1). The posttests were conducted at 20 minutes after exposure to PM2.5 on day 9 and day 18. On day 19, the mice were anesthetized with 2.5% Avertin (Sigma-Aldrich, St. Louis, MO, USA) at 400 mg/kg by intraperitoneal injection for tear ferning tests and ocular surface photography. The photographs were assessed later for ocular surface indexes, including smoothness,²⁸ opacity,²⁹ topography, and lissamine green stain³⁰ following the criteria used in previous publications. The mice were then killed to collect eyes with their periocular tissues for further analyses.

Clinical AC Scoring

The major clinical AC manifestations including eyelid edema, tearing, and scratching were scored according to previously published criteria.^{7,31,32} Eyelid edema and tearing were graded from 0 (absent), 1 (mild), 2 (moderate) to 3 (severe). For scratching behavior assessment, the mice were isolated from each other and placed in a cage individually for at least an hour to adapt to the environment. A round of scratch was defined as each hindlimb reaching the eye and returning to the ground. The scratching behavior was video recorded for counting, and the average scratch rounds in 30 seconds were calculated.³³ An average score from 10 mice was obtained for eyelid edema, tearing, and scratching behavior for each study group, respectively. Then, a total score for each study group was obtained by summing up the average scores.

Tear Volume

Tear volume was measured with phenol red thread test (Zone-Quick; Lacrimedics, Eastsound, WA, USA), as described previously.³⁴ Mice were kept immobile by intraperitoneal injection of Avertin 400 mg/kg. The lower eyelid was pulled down slightly, and a 1-mm portion of the thread was placed on the lower palpebral conjunctiva for 20 seconds. The length of the moistened fragment was measured. Measurements were obtained for both the right and the left eyes.

Tear Breakup Time (TBUT)

Tear breakup time (TBUT) assessments were performed by dropping 5 μL 1% fluorescein in 0.9 NaCl saline on the eye surface using a micropipette. After three aided blinks, the ocular surface was video recorded under a fluorescence microscope for later evaluation.^{29,35}

Tear Ferning

Tear ferning was performed and assessed according to previously published procedures.³⁶⁻³⁸ A 2- to 4-μL aliquot of 0.9% NaCl was dropped to the eye to wash the ocular surface six or seven times using a micropipette. The aliquot was then transferred and spread onto a glass slide. The preparation was air-dried under room temperature and humidity to allow ferning formation.

Histologic Analyses

After mice were killed by carbon dioxide, the tissues including eye, eyelid, meibomian, and lacrimal glands were collected,

TABLE. Results of Clinical Allergic Conjunctivitis Scoring

| Day | Symptom | Blank | 3.2 mg/mL | 6.4 mg/mL | 12.8 mg/mL | 12.8 mg/mL + AT |
|--------|--------------|-------|-----------|-----------|------------|-----------------|
| Day 0 | Eyelid edema | 0 | 0 | 0 | 0 | 0 |
| | Tearing | 0.1 | 0.2 | 0.4 | 0.1 | 0.3 |
| | Scratching | 0.4 | 0.3 | 0.3 | 0.4 | 0.3 |
| | Total | 0.5 | 0.5 | 0.7 | 0.5 | 0.6 |
| Day 9 | Eyelid edema | 0.8 | 1.8* | 1.5 | 2.0* | 2.0* |
| | Tearing | 0.3 | 0.7 | 0.7 | 0.9 | 1.1* |
| | Scratching | 0.1 | 0.2 | 0.6 | 0.6 | 0.6 |
| | Total | 1.2 | 2.7 | 2.8 | 3.5 | 3.7 |
| Day 18 | Eyelid edema | 0.7 | 1.7* | 1.7* | 1.6* | 1.8* |
| | Tearing | 0.3 | 0.9 | 0.8 | 1.1* | 2.0* |
| | Scratching | 0.5 | 1.3 | 1.4 | 1.1 | 1.2 |
| | Total | 1.5 | 3.9 | 3.9 | 3.8 | 5 |

* $P < 0.05$, compared with blank group.

fixed, and embedded into paraffin wax. Tissue sections in 5- μ m thickness were cut with a sliding microtome. The sections were mounted on glass slides and stained with hematoxylin-eosin (HE) to observe the structure changes in cornea and conjunctiva. Periodic acid Schiff (PAS) stain was used to evaluate the goblet cells and Giemsa stain was performed to detect the infiltrated eosinophils.

Statistical Analyses

The results of clinical AC scores were analyzed by Mann-Whitney U test. All the other results were analyzed by ANOVA on the SPSS 18 package (SPSS, Inc., Chicago, IL, USA). Significance was expressed by either $P < 0.05$ or $P < 0.001$. All quantifications were performed through triple repeats by three individuals without knowledge of the study groups.

RESULTS

Heavy Metal Composition in the Collected PM2.5 Samples

The composition of heavy metals (i.e., V, Pb, Cu, Cd, Zn, Cr, Sb, Ni, Co, As, and Mn) in the PM2.5 collected in October,

November, and December 2017 was measured. Among the heavy metals detected, Fe, Cu, Zn, Pb, and Mn were the most prominent, with concentrations of 1.49, 0.31, 0.78, 74.60, and 0.16 μ g/mL, respectively.

Effects of PM2.5 on Clinical AC Scores

Clinical AC symptoms, including eyelid edema, tearing, and scratching on days 0, 9, and 18, were scored. No apparent baseline difference in total scores (range, 0.5–0.7) among all study groups was observed on day 0 (Table), although minor deviations in individual symptoms existed. Noticeably, there was no eyelid edema in all study groups on day 0.

On day 9, evident increase of all individual scores, including eyelid edema scores, was observed, leading to increased total scores in all study groups. The total scores were increased dose-dependently. The highest total score was 3.5 with PM2.5 at 12.8 mg/mL, compared with the score at 1.2 (blank), 2.7 (3.2 mg/mL), and 2.8 (6.4 mg/mL). Interestingly, when the eyes were exposed to PM2.5 at 12.8 mg/mL, followed by treatment with AT, the total score was even increased to 3.7, indicating that AT did not help to ameliorate the situation.

On day 18, a more evident increase in scores in all three symptoms was found in the study groups exposed to PM2.5, in contrast to the only slight increase in scratching in the blank

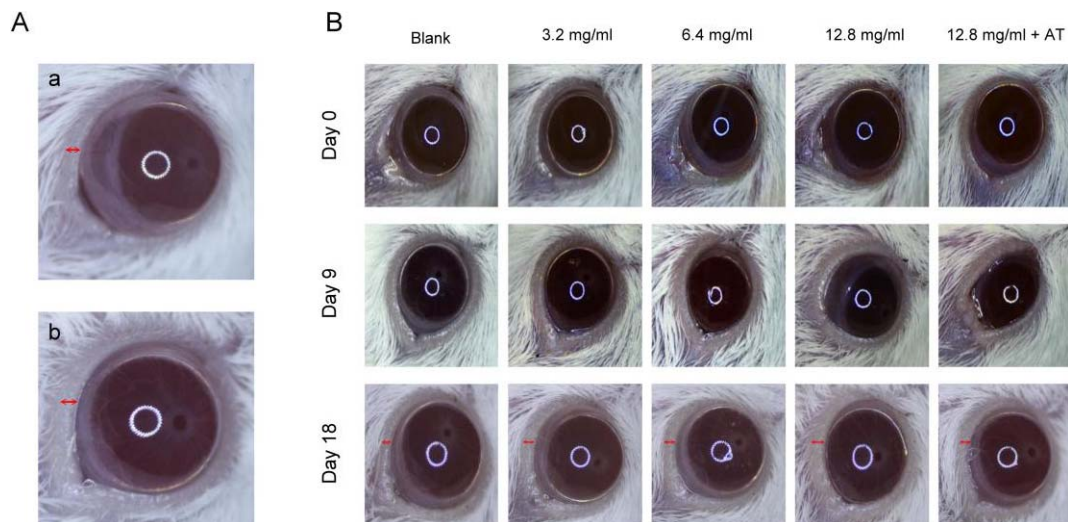


FIGURE 1. (A) An example of a mouse eye 20 minutes after exposure to 0.9% NaCl (a) and PM2.5 in 0.9% NaCl (b). Notice the more evident eyelid edema (red arrow) in (b). (B) The extent of eyelid edema on days 0, 9, and 18 after exposure to 0.9% NaCl (blank) or PM2.5 in 0.9% NaCl at 3.2, 6.4, 12.8, and 12.8 mg/mL with AT treatment.

Acute Allergic Conjunctivitis Induced by PM2.5

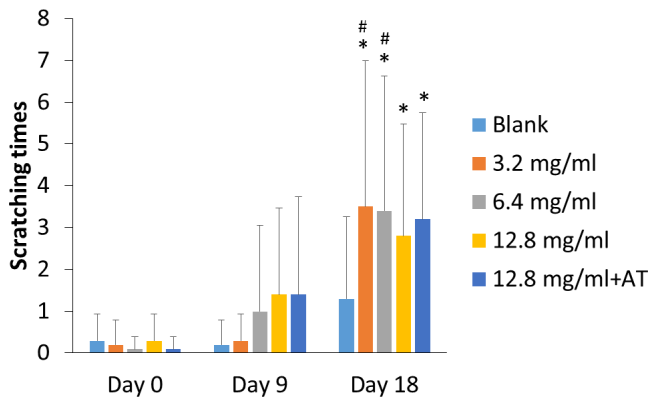


FIGURE 2. Scratching times of blank control and experimental mice exposed to PM2.5 at 3.2, 6.4, 12.8, and 12.8 mg/mL treated with AT at days 0, 9, and 18. * $P < 0.05$, compared with day 0; # $P < 0.05$, compared with day 9.

group. The total score was 1.5 in the control group, far less than the total scores around 3.8 to 3.9 in the study groups. In all groups, the increase in scratching contributed to most of the total score increase. Treatment with AT after exposure to PM2.5 did not help to alleviate the symptoms, with a total score at 5.0—even worse than without treatment.

An example of eyelid edema on day 9 after PM2.5 exposure (Fig. 1Aa), in contrast to the normal status from the blank control group (Fig. 1Ab), is shown in Figure 1. The blank control eye exhibited normal eyelid width (indicated by red arrow), evidently different from the PM2.5-treated eye with widened eyelid due to edema. The eyelid edema initially observed on day 9 became worse on day 18 in all study groups (Fig. 1B) except for the control group. Furthermore, a trend of dose-dependent severity of eyelid edema was observed and treatment with AT did not alleviate eyelid edema.

On day 18, scratching times were significantly increased in all groups exposed to PM2.5, compared with those prior to exposure on day 0 and those on day 9 (Fig. 2), indicating a trend of increasing scratching times from day 0 to day 9, and further to day 18. Again, treatment with AT did not apparently help to reduce the scratching behavior.

Temporarily Enhanced Tear Secretion and Longer TBUT With PM2.5 Exposure

On day 9, after PM2.5 exposure, the tear volume was significantly increased in the 12.8 and the 12.8 mg/mL + AT groups compared to the blank control group (Fig. 3). Nevertheless, the tear volume increase was only temporary, as no significant difference was observed between all the other study groups and the blank control group on day 18.

TBUT was significantly longer in the 6.4, 12.8, and 12.8 mg/mL + AT groups on day 18 than that in the blank control group (Fig. 4).

Since the tear quantity and quality assays showed results that were different from dry eye symptoms, to further characterize the effects of PM2.5 exposure, we also performed corneal surface photography analyses after treating with PM2.5 for 18 days. The results showed no difference in transparency, smoothness, topography, or lissamine green staining on the ocular surface in all groups (data not shown). In addition, histologic analyses did not show evident changes in cornea structures (data not shown).

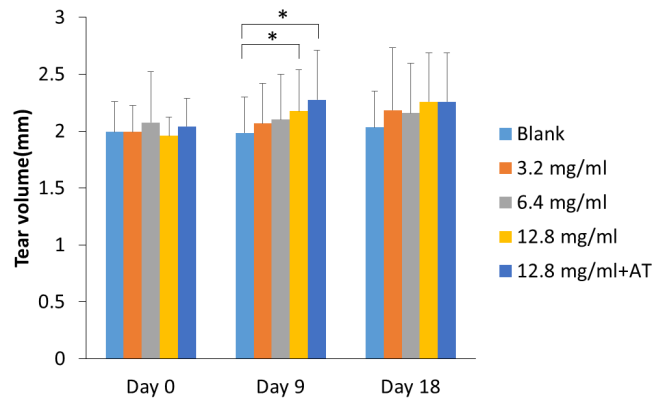


FIGURE 3. Mean tear volume of the blank control and experimental mice exposed to PM2.5 at 3.2, 6.4, 12.8, and 12.8 mg/mL treated with AT at days 0, 9, and 18. * $P < 0.05$, compared with the blank control group.

Effects of PM2.5 on Tear Ferning

A trend of less tear fern crystal formation was observed after exposure to PM2.5 under increasing concentrations (Fig. 5). Note that when PM2.5 at 12.8 mg/mL was applied, the crystallization of tear ferns was severely blocked and treatment with AT further impeded tear ferning formation.

Upper and Lower Palpebral Conjunctiva Goblet Cell Changes Under the Influence of PM2.5

The results of PAS stain showed a dose-dependent increase of goblet cells in the upper palpebral conjunctiva in the PM2.5-treated groups, compared with the blank group (Fig. 6A). In contrast, in the lower palpebral conjunctiva, a dose-dependent decrease of goblet cells was found (Fig. 6B). However, neither the increase nor the decrease of goblet cells was statistically significant. Despite that, treatment with AT appeared to ameliorate the changes in goblet cells.

PM2.5-Induced Eosinophil Cell Infiltration in Upper and Lower Palpebral Conjunctivas and in Meibomian Glands

Eosinophil cell infiltration, a major hallmark of AC, was detected by Giemsa stain, and the results showed extensive

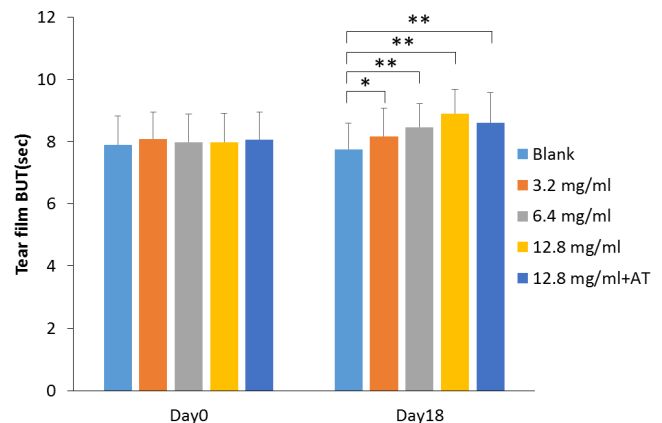


FIGURE 4. Tear film breakup time (TBUT) of blank control and experimental mice exposed to PM2.5 at 3.2, 6.4, 12.8, and 12.8 mg/mL treated with AT at days 0, 9, and 18. * $P < 0.05$; ** $P < 0.001$, compared with blank group.

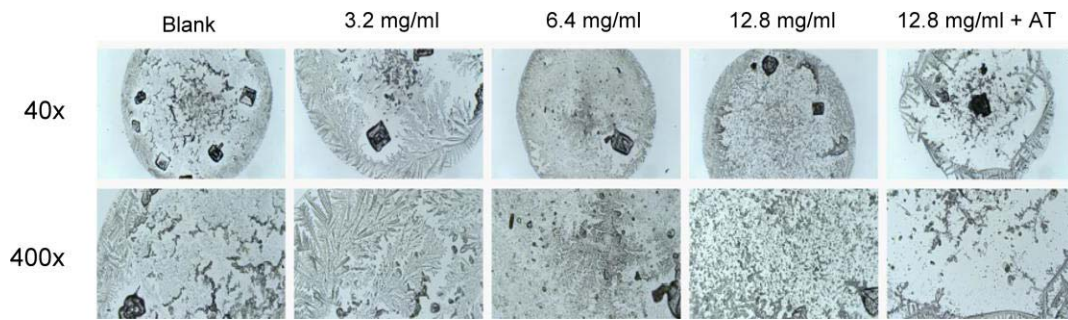


FIGURE 5. Light micrographs of tear ferning in the blank control and experimental mice exposed to PM2.5 at 3.2, 6.4, 12.8, and 12.8 mg/mL treated with AT on day 19 (magnification at $\times 40$ and $\times 400$, respectively).

eosinophil cell infiltration as the PM2.5 doses were increased (Fig. 7A). Unlike the differential effects of PM2.5 on goblet cells between the upper and lower palpebral conjunctiva, both the upper and lower conjunctivas exhibited increased eosinophil infiltration in a dose-dependent manner (Fig. 7B). Dose-dependent increase of eosinophil infiltration was also detected in the meibomian glands (Fig. 8).

DISCUSSION

Even though AC has been previously induced in mice, rats, and guinea pigs, the animal AC models are created through

injection of allergen mixed with adjuvant, followed by subsequent conjunctival challenge with the same solution.^{31,39} To date, whether an AC animal model may be created solely through direct allergen contact with the ocular surface remains unclear.

The effects of airborne particulate matter, including PM2.5, on the ocular surface have been investigated in cell lines¹²⁻¹⁵ as well as in clinical studies.^{16,17} The main findings were detrimental effects to the corneal epithelium¹²⁻¹⁴ through increased production of proinflammatory cytokines and changes in tear osmolarity.¹⁶ The only animal studies addressing PM2.5-induced effects on the cornea that have been reported so

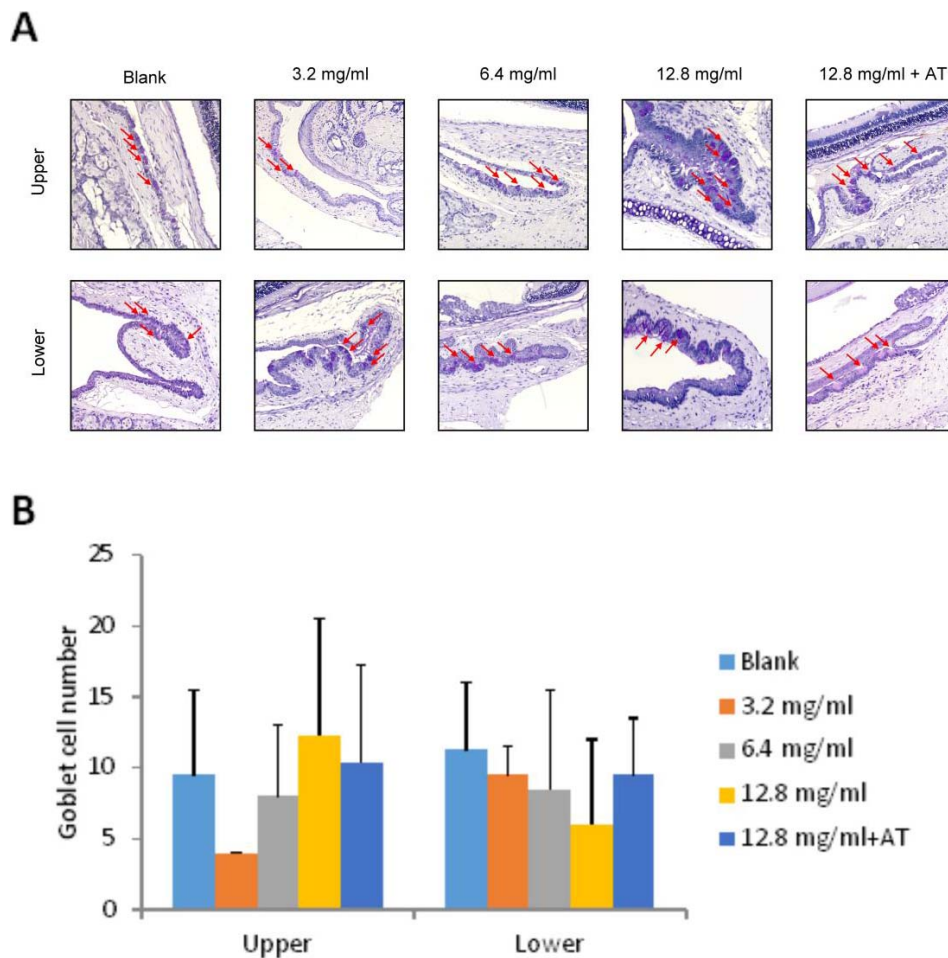


FIGURE 6. (A) Goblet cells detected by periodic acid Schiff (PAS) stain on conjunctival tissues from the blank control and experimental mice exposed to PM2.5 at 3.2, 6.4, 12.8, and 12.8 mg/mL treated with AT on day 19. (B) Quantification of goblet cells in the upper and the lower conjunctiva (magnification at $\times 100$).

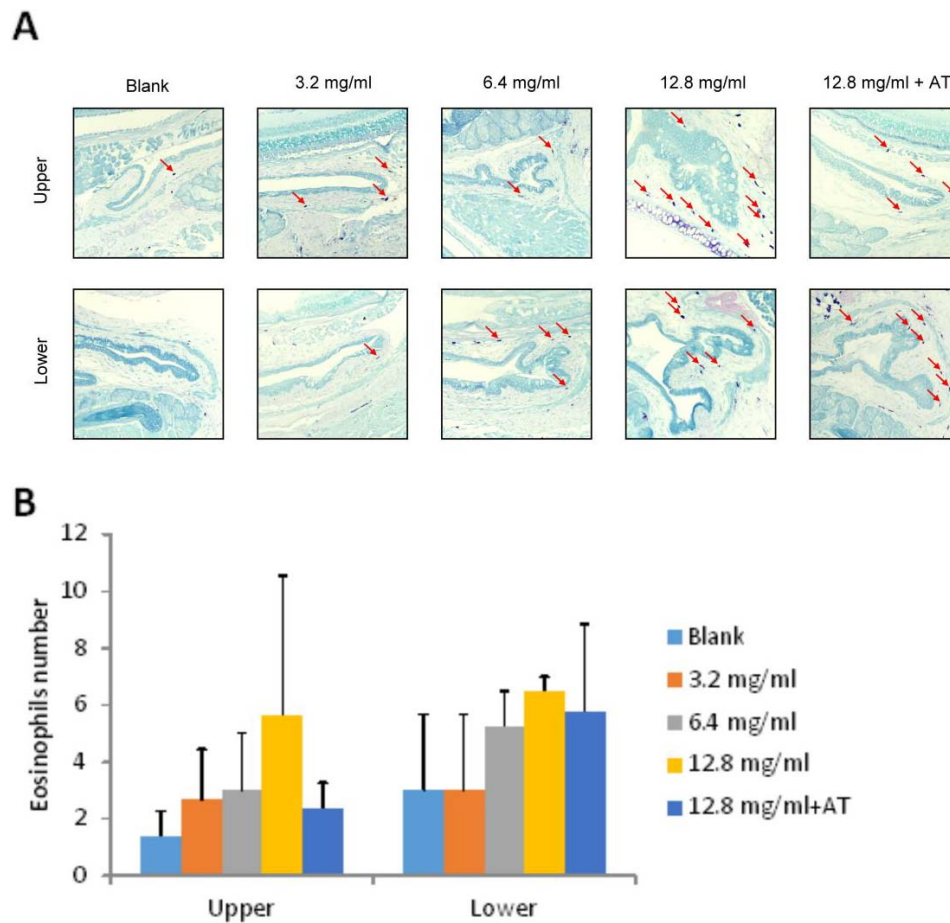


FIGURE 7. (A) Eosinophils detected by Giemsa stain on conjunctival tissues from the blank control and experimental mice exposed to PM2.5 at 3.2, 6.4, 12.8, and 12.8 mg/mL treated with AT on day 19. (B) Quantification of eosinophils in the upper and the lower conjunctiva (magnification at $\times 100$).

far lead to evidence of PM2.5 interference in cornea epithelium wound healing¹⁸ and induction of dry eye syndrome.¹⁹ Hence, all previous reports regarding PM2.5 effects on the ocular surface implicated only dry eye conditions, without referring to AC pathogenesis. There has been no report on whether PM2.5 may induce AC through its natural route, that is, direct contact with the ocular surface, and no report of an AC animal model induced by PM2.5 has been published.

Nevertheless, PM2.5 has been generally known to contain allergens to induce allergic reactions.^{25,26} In addition, many lines of epidemiologic evidence support the concept that PM2.5 may pose a risk to induce AC.^{4,20-22,40} These previous reports justify the hypothesis that the ocular surface directly exposed to PM2.5 may lead to AC pathogenesis. The results of the present study demonstrated that AC conditions can be induced by PM2.5 within a relatively short period of time. To

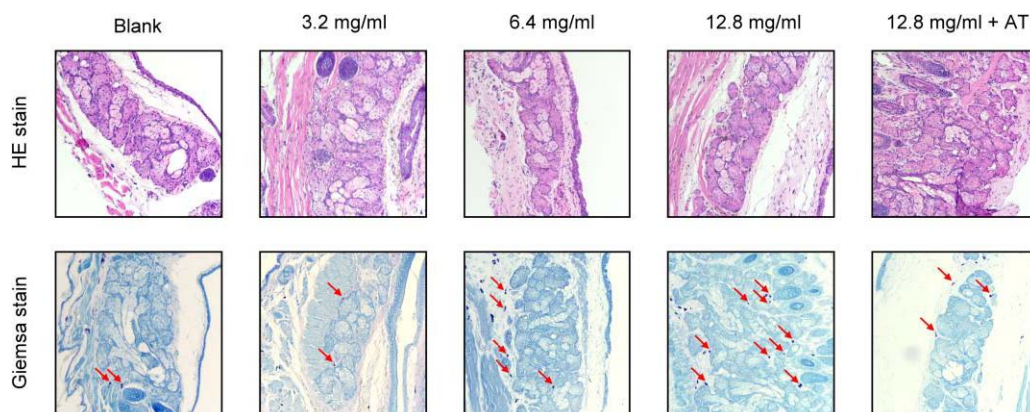


FIGURE 8. Hematoxylin-eosin stain and Giemsa stain of meibomian glands from the blank control and experimental mice exposed to PM2.5 at 3.2, 6.4, 12.8, and 12.8 mg/mL treated with AT on day 19 (magnification at $\times 100$).

our knowledge, this is the first animal AC model induced by PM2.5 through direct PM2.5 application on the ocular surface.

Interestingly, not all of our results were consistent with previous findings by Cui et al.¹⁸ and Tan et al.¹⁹ in support of PM2.5-induced dry eye status. The clinical AC scoring results may share some common characteristics with dry eye symptoms, such as eyelid edema and tearing as early signs of dry eye. Apart from that, the discomfort of dry eye conditions may also induce scratching behavior. On the contrary, the present data showed a temporary enhancement of tear volume and longer TBUT with PM2.5 exposure; both are contradictory to typical dry eye pathogenesis. These different results, as compared to those published by Cui et al.¹⁸ and Tan et al.,¹⁹ may be derived from several deviations: Firstly, PM2.5 components are generally complicated mixtures and vary considerably depending on season, region, and resources. For example, the PM2.5 used in this study contained much less Mn (0.17 vs. 0.35 µg/mL), Fe (1.49 vs. 2.83 µg/mL), and Zn (0.78 vs. 4.65 µg/mL) than those in the PM2.5 used by Cui et al.¹⁸ Secondly, different durations of PM2.5 treatments may trigger distinct outcomes. Cui et al.¹⁸ exposed the mouse ocular surface to PM2.5 for 7 days and Tan et al.¹⁹ for 14 days, in contrast to 19 days in the present study. Thirdly, there may be dose-dependent reactions with different concentrations of PM2.5. We applied PM2.5 with concentrations ranging from 6.4, 12.8, and 12.8 mg/mL, while 100 µg/mL was used by Cui et al.¹⁸ and 5 mg/mL was used by Tan et al.¹⁹

Apart from the temporary enhancement of tear volume and longer TBUT, some critical features distinguish the PM2.5-induced AC status in this study from dry eye conditions. With increasing concentrations of PM2.5, no apparently more severe ocular surface damage was observed, which is not typical of dry eye induction. Besides, AT treatments usually ameliorate eyelid edema, tearing, and scratching with dry eye. Nevertheless, our results showed an even higher total clinical score than without treatment. Furthermore, the number of goblet cells was increased with higher PM2.5 doses, at least in the upper conjunctiva, contrary to the typical decrease of conjunctival goblet cells under dry eye conditions. In addition, tear ferning blockage under the influence of higher PM2.5 doses also indicates that our model is more likely an AC model. Dry eye conditions usually come with higher tear osmolarity, enhancing crystal formation from the tear components. The results showed less tear ferning formation in the group with AT treatment, even though PM2.5 at 12.8 mg/mL was applied. This blockage of tear ferning formation is likely due to washing away of tear salts by AT, resulting in lower tear osmolarity and thus less tear ferning. The fact that tear ferning was also blocked in the group with PM2.5 at 12.8 mg/mL but without AT treatment, indicates that it is not a case of dry eye condition. The most important feature that distinguish our AC model from the dry eye model is the extensive eosinophil infiltration in conjunctiva and in meibomian glands, which is the hallmark of AC, unlike the dry eye conditions in which neutrophils are usually the major infiltrating leukocytes.

The results of this study extend the spectrum of aftermaths that may be observed with direct PM2.5 exposure on the ocular surface. As PM2.5 components vary considerably, it is difficult to shape systematic studies, and low comparability will be inevitable among different studies. Despite the limitations, some insights may still be derived from the present study. For example, the differential goblet cell changes between upper and lower palpebral conjunctiva indicates that PM2.5 doses may critically determine the effects. The upper conjunctiva tends to be more washed by tear flow, in contrast to the lower conjunctiva where more pollutants usually accumulate due to

gravity. It is likely that PM2.5 will accumulate more in the lower conjunctiva, leading dose-dependently to the reduction of goblet cells. Another more important insight is that efforts should be made by clinicians toward early differential diagnosis between AC and dry eye, since AT treatment did not ameliorate AC in our results.

The current results showed longer TBUT in all study groups exposed to PM2.5 on day 18 of the experiment, as compared to that of the control group. This finding is contrary to some previous studies that reported tear instability associated with seasonal AC.^{41,42} The difference is presumably due to the differential effects between PM2.5 and other allergens, as it was reported that seasonal allergic inflammation did not cause permanent tear film instability outside the pollen season.⁴³ Alternatively, the effects of an allergic induction in the acute phase are likely to differ from those long-term consequences. With long-term AC, tear film stability is usually impaired as a result of reduced conjunctival goblet cell density, often concurrent with decreased tear secretion, making it very difficult to perform differential diagnosis between AC and dry eye.^{44,45} Unlike typical symptoms of dry eye and long-term AC, our results showed longer TBUT, higher upper conjunctiva goblet cell density, and no apparent decrease in tear secretion on day 18 of the experiment. Therefore, the mouse AC model produced in the present study is better regarded as an AC model in the acute phase.

There are, however, several modifications that can be performed to improve the current mouse AC model induced by PM2.5. Firstly, aerosol samples, ozone, for example, have been applied in a gas chamber to induce an inflammatory response on the ocular surface.⁴⁶ Likewise, aerosol PM2.5 may also be applied in a gas chamber to better imitate the natural situations. Secondly, even though scratching is considered a clinical symptom to reflect AC severity, it is a common feature shared by dry eye status. In addition, scratching behavior in mice can be easily affected by extrinsic sounds or environmental unfamiliarity³³ and by place aversion.⁴⁷ These interfering factors have limited the use of scratching behavior as an AC indicator. Therefore, other behavior indexes may be used. For example, reduced activity has been reported with severe AC.⁸

Given that numerous reports have confirmed that exposure to ambient PM2.5 will lead to increased health risks,⁴⁸ more people become alerted to air quality and carry masks, and thus reduce the amount of allergens inhaled into the respiratory system. Eye goggles are far less commonly used for prevention against direct PM2.5 contact with the ocular surface. Therefore, increasing PM2.5 in the atmosphere will lead to more AC prevalence in the future, demanding more research. The establishment of the PM2.5-induced acute AC mouse model in the present study will facilitate further research.

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