

Exposure of PM2.5 Exacerbates COVID-19 In Vivo

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Abstract: Coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) affects many tissues and organs, especially lung damage. The respiratory system related comorbidities can increase the COVID-19 clinical symptoms and cause more damages. Since high PM2.5 exposure decreases the respiratory system function, I hypothesize that PM2.5 can induce aggravated COVID-19 symptoms. I propose to construct the human angiotensin converting enzyme 2 (hACE2) knock in mice model to mimic COVID-19 patients. PM2.5 particulates will be delivered into the mice container in the aerosol form for consecutive 5 days ending on day -7 (Day 0 is the day of SARS-CoV-2 virus challenge). On day 21, we will sacrifice the mice and analyze the autopsy samples via ELISA, flow cytometry and histology assays and so forth, which determine the secretion level of inflammatory cytokines and characterize the function of related tissue or organs. It will be shown that pre-PM2.5 exposure induces more severe COVID-19 symptoms with highly activated inflammatory responses.

1. INTRODUCTION

A cluster of COVID-19 cases were first reported in Wuhan, China in December 2019. In spite of the fact that most COVID-19 patients experience mild symptoms, COVID-19 can still cause death especially to those patients with preexisting comorbidities. Due to its rapid spreading, COVID-19 was the third leading cause of death in 2020. And among COVID-19 patients who died, 83.29% had a preexisting comorbidity, as stated in the statistics [2]. Besides, the current understanding of sequelae is limited. It is not quite clear of its prolonged effects on recovered patients even for those with mild symptoms. Pneumonia is also seen among the acute COVID-19 patients since the major target area attacked by SARS-CoV-2 virus is lung. Another severe complication is COVID-19 sepsis due to uncontrolled immune response after infection. Moreover, the lung plays an important role in the circulation system through the blood vessels, once the virus goes into the bloodstream, the affected area will be enlarged along with virus migration.

Several studies have proved the association between air pollution and COVID-19[1]. Given the fact that preexisting health conditions in the respiratory system can increase the severity of COVID-19 and PM2.5 is a major indicator of polluted air with the result of damaged lungs. I propose that PM 2.5 exposure induces more severe COVID-19 symptoms.

This project is the first time to explore the impact of PM2.5 exposure prior to COVID-19 infection. It has strong significance because it gives people an effective

method via reducing the air pollution to decrease the events of acute or severe COVID-19 cases.

2. METHODS

2.1. PM2.5 Aerosol Preparation

We plan to harvest the PM2.5 from the environment with heavy traffic. We expose a Polytetrafluoroethylene (PTFE) attaching paper to collect the particulates with 2um diameter as the source of PM2.5 for our project. Next step is scraping these particulates to another filter paper. Water is added to wash out particulates using suction filtration. Then, the particulates are dried until water is completely removed. Lastly, the particulates are dissolved in Cyrene to 20mg/mL for later use. We plan to utilize propellants and the evaporation function of Cyrene to make PM2.5 into the form of particles again in the aerosol before mice breathing in. PM2.5 particulates are delivered into the mice container for consecutive 5 days ending on day -7 (Day 0 is the day of SARS-CoV-2 virus challenging).

2.2. Human ACE2 Knock in Mice Model

Because the target for SARS-CoV-2 attacking is human angiotensin-converting enzyme II (ACE2) in human bodies, C57BL/6 mouse model expressing human ACE2 (hACE2) by using CRISPR/Cas9 knockin technology will be an accurate model to mimic COVID-19 patients. We use western blot assay to verify hACE2 expression in the mice. WT C57BL/6 mice will be used as the control

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group. On day 21, we will sacrifice the mice and analyze the autopsy samples via diverse assays.

2.3. SARS-CoV-2 Propagation and Titration

SARS-CoV-2 strain was originally isolated by a COVID-19 patient. The virus was amplified on Vero cells and titrated by standard plaque forming assay. Briefly, Vero cells in 12-well plates were infected with a 10-fold serial dilution of viruses. The plates were incubated at 37C for 1 h and cells were overlaid with 1% low-melting point agarose in DMEM containing 2% FBS. After further incubation at 37C for 2 days, the cells were fixed with 4% formaldehyde and stained with 0.2% crystal violet to visualize the plaques. All experiments involving infectious SARS-CoV-2 were performed in biosafety level 3 containment laboratory.

2.4. Mice groups and SARS-CoV-2 virus challenge

hACE2 mice were intranasally infected with 4e5 plaque-forming units (PFU) of SARS-CoV-2, and WT C57BL/6 mice that received the same dose of viral challenge were set as control. For each group, we also divided them into w/ and w/o exposure to PM2.5 particulates. Monitoring parameters after exposure and challenge include body weight and observable clinical symptoms.

2.5. ELISA

TNF- α , TGF- β 1 and IL-6 secretion levels were detected by ELISA. Serum samples were incubated in 96-well ELISA plates with primary antibodies. After the addition of biotinylated antibodies, the plates were washed and reacted with HRP-conjugated streptavidin. A microplate reader was used to measure the results.

2.6. Western Blot

Mouse tissues were homogenized in Cell lysis buffer for Western and IP (20 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 100 mM EDTA, and 1% Triton X-100) supplemented with Phenylmethanesulfonyl fluoride. The denatured protein lysates were separated using 10% SDS-PAGE gels. After transfer, human ACE2 antibody (1:1000) will be added, followed by horseradish peroxidase (HRP)-conjugated anti-human IgG (1:2,000). Expose the film under the appropriate condition.

2.7. SARS-CoV-2 RNA RT-qPCR quantification

The viral RNA quantification was performed by RT-qPCR targeting the S gene of SARS-CoV-2. RT-qPCR was performed with the following primers and probes: CoV-F3, CoV-R3 and CoV-P3.

2.8. Flow Cytometry

Perform bronchoalveolar lavage for the corresponding tissues, we will use the flow cytometry machine to detect numbers of granulocytes, monocytes and eosinophils using specific antibodies with different fluorescence dyes for all groups.

2.9. Histopathology

Mouse tissues were excised and fixed with 10% neutral buffered formaline, dehydrated and embedded in paraffin. Each embedded tissue was sectioned into 4 mm thickness longitudinal sections. Three tissue sections derived from different parts of each tissue were stained with hematoxylin and eosin (H&E) according to standard procedures for examination by light microscopy. The degree of lung damage under the light microscopy was assessed by the degeneration of alveolar epithelial cells, the expansion of parenchymal wall, edema, hemorrhage, and inflammatory cells infiltration.

Table 1: Results

The impact of PM2.5 on hACE2 KI mice after SARS-CoV-2 infection	Result 1	Result 2	Result 3	Result 4	Result 5	Result 6
Increased expression of inflammation cytokines (TNF- α , TGF- β 1 and IL-6) in serum	+	+	+	+	+	-
Increased viral load in the affected tissues [^]	+	+	+	+	-	-
Decreased body weight with more severe clinical symptoms*	+	+	+	-	-	-

increased infiltrating granulocytes, monocytes and eosinophils from bronchoalveolar lavage	+	+	-	-	-	-
More damages to the lung tissue observed via histopathological assay	+	-	-	-	-	-
Increased the viral load in the non-affected tissues [□]	-	-	-	-	-	-

+ means statistically significant increased, - means no statistically significant changes compared with control.

Table 1-Possible results. Corresponding samples will be collected from the hACE2 KI mice group after SARS-CoV-2 infection with PM 2.5 intervention based on different assays. The negative control is the hACE2 KI mice group after SARS-CoV-2 infection without PM 2.5 intervention.

△ Affected tissues include liver, spleen, small intestine, ovary, and brain.

*observable clinical symptoms include lethargy, reduced activity and increased respiration rate.

□ non-affected tissues include bladder, thyroids, and bones.

Possible result 1: High PM2.5 increases the level of inflammation related cytokines and immune cells. SARS-CoV-2 viral load is also elevated while not generating effects in the non-targeted areas. Decreased mice body weight is also observed in the PM2.5 intervention group.

COVID-19 symptoms increase via the mechanism of elevated inflammation cytokines in serum, infiltrating granulocytes, monocytes and eosinophils in the alveoli area. It is detected that SARS-CoV-2 viral load increases in the tissues with rich hACE2 expression, like liver, spleen, small intestine, ovary, and brain. However, there are no significant viral load increases in the non-targeted areas, including bladder, thyroids, and bones. Body weight of mice with PM2.5 intervention gradually decreased over time due to more severe symptoms. And, for this group, there are more physical damages to the tissues observed by histopathological assay. Possible result 1 concludes that PM2.5 increases the severity of COVID-19 disease while not affecting the non-targeted areas.

Possible result 2: PM2.5 intervention significantly increases generation of inflammation cytokines in mice serum after SARS-CoV-2 infection. Consistent with this result, increased viral load, inflammatory immune cells and clinical symptoms are also found. For this possible result 2, physical organ damages are not observed.

It is detected that PM 2.5 increases the viral load in the tissues such as liver, spleen, small intestine, ovary, and brain. The body weight of the mice decreases with more severe clinical symptoms. PM 2.5 significantly increased infiltrating granulocytes, monocytes and eosinophils from the samples through bronchoalveolar lavage. However, there is no significant physical damage to the tissues. Also, There is no increase in the viral load of the non-affected tissues.

Possible result 3: SARS-CoV2 infection leads to elevated inflammation cytokine production in the mice serum of high PM2.5 group compared with control group. Decreased mice body weight was observed along with the increased inflammation cytokine. SARS-CoV-2 virus replicates more in the affected areas of this experimental group.

Serum inflammation cytokines including TNF- α , TGF- β 1 and IL-6 are generated more in the high PM2.5 group after virus challenge. Virus has more replicates in this group along with the decreased mice bodyweight. However, there are no significant changes regarding inflammatory immune cell number and S protein staining positive signals between experimental and control mice groups. Possible result 3 proves that high PM2.5 increases the severity of COVID-19 disease via elevated serum inflammation cytokines, more replicated virus in the lung instead of obvious increases of inflammatory immune cells. Physical organ damages are found in the experimental group. For the non-targeted tissues, sample analysis results indicate they are protected from virus infection.

Possible result 4: PM2.5 intervention mice group shows elevated level of serum inflammation cytokines. In the areas attacked by SARS-CoV-2, the virus replicates more compared with the control group.

High PM2.5 leads to the increasing generation of serum inflammatory cytokines including TNF- α , TGF- β 1 and IL-6. Besides, the more replicated viruses show in the targeted areas due to inhalation of more PM2.5 particulates. However, there are no significant changes in the mice's body weight. No obvious inflammatory immune cell number changes are found. Compared with the control group, organ physical function has no differences between the experimental group and control group. Again, for the non-targeted tissues, sample analysis results indicate there is no virus infection.

Possible result 5: Serum inflammation cytokine level is detected to increase for the hACE2 KI mice after virus infection. All the other assays have the negative results.

The only positive result is increased serum inflammation related cytokines including TNF- α , TGF- β 1 and IL-6. On the contrary, the inflammatory immune cell number and physical organ function is not changed. The obvious virus load is not detected. Furthermore, along with no observable clinical symptoms, mice body weight does not drop over time.

Possible result 6: All abnormalities in histology or inflammation response on cellular or molecular levels are not found.

All results of six designed assays are negative which means that the experimental group with PM_{2.5} intervention has no significant inflammation response, obvious clinical symptoms or decreased tissue physical function. PM_{2.5} does not affect the symptom severity of COVID-19 diseases caused by SARS-CoV-2 viruses.

3. DISCUSSION

As the first project to explore the impact of PM_{2.5} exposure prior to COVID-19 infection, the possible result 1 shows the clinical significance of this topic to give people an idea of decreasing the severe COVID-19 cases via protecting the air quality.

It has been observed that when the air quality index (AQI) increases by 10 units, the spreading rate of the coronavirus increases by 5-7%. Another study has shown that pM 2.5 and pM 10 could increase vulnerability to COVID-19. Those findings inspire our future research topic of studying PM_{2.5} as a carrier of SARS-CoV-2 virus to influence COVID-19 spreading speed and risks of infection within polluted environments.

One limitation of this project is that it does not reflect on the age factor. It is known that COVID-19 symptom severity and death rate is stratified between different age groups. We plan to continue the research of bifurcating the mice into young age and old age to explore this age factor targeting COVID-19.

Because there are no available publications supporting the exact PM_{2.5} exposure amount to cause the physical lung damages, we might meet the unexpected condition which lacks preexisting lung damages before COVID-19 infection. As for possible result 6, if there is no significant association between PM_{2.5} pre-exposure and virus challenge, we could do virus challenge first followed by PM_{2.5} exposure when the symptoms show up.

4. CONCLUSION

The hACE2 knock in model successfully expresses the hACE2 in the mice verified by the western blot assay. Since SARS-Cov-2 enters the host cell via hACE2 receptor and damages the cell later, this hACE2 knock in mice is an accurate model of COVID-19 infection in humans.

Through this proposed project, we explore the association between PM_{2.5} exposure prior to COVID-19 infection. The possible result 1 shows that PM_{2.5}, a factor adversely affecting the preexisting respiratory system condition, increases the COVID-19 symptoms afterwards showing highly activated immune response by increasing the secretion of inflammatory cytokines and generation of inflammatory cells. More virus replication and severe physical damages are also proved within the experimental group compared with the control group.

There is a possibility that more COVID-19 variants will occur in the future. It is proved that PM_{2.5} exposure will increase the COVID-19 symptoms from possible result 1. This project will let us know that reducing air

pollution is a potential measure of preventing the occurrence of the next pandemic.

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