SIRT1 Protects Against Particulate Matter-Induced Oxidative Stress in Human Corneal and Conjunctival Epithelial Cells

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Purpose. Sirtuin1 (SIRT1) as a hot therapeutic target for oxidative stress–associated diseases that has been extensively studied. This study aimed to determine the changes in SIRT1 expression in particulate matter (PM)–induced corneal and conjunctival epithelial cell damage and explore potential drugs to reduce PM-associated ocular surface injury.

Methods. Immortalized human corneal epithelial cells (HCECs) and human conjunctival epithelial cells (HCjECs) were exposed to an ambient PM sample. Cytotoxicity was evaluated by water-soluble tetrazolium salt–8 assay. SIRT1 expression was measured by Western blot analysis. Reactive oxygen species (ROS) production, cell apoptosis, mitochondrial function, and cell senescence were assessed by using 2′,7′-dichlorofluorescein diacetate assay, annexin V apoptosis assay, tetramethylrhodamine ethyl ester assay, and senescence β-galactosidase staining, respectively.

Results. PM-induced cytotoxicity of HCECs and HCjECs occurred in a dose-dependent manner. Increased ROS production, as well as decreased SIRT1 expression, were observed in HCECs and HCjECs after 200 µg/mL PM exposure. In addition, PM induced oxidative stress-mediated cellular damage, including cell apoptosis, mitochondrial damage, and cell senescence. Interestingly, SRT1720, a SIRT1 activator, increased SIRT1 expression and decreased ROS production and attenuated PM-induced cell damage in HCECs and HCjECs.

Conclusions. This study determined that SIRT1 was involved in PM-induced oxidative stress in HCECs and HCjECs and found that ROS overproduction may a key factor in PM-induced SIRT1 downregulation. The SIRT1 activator, SRT1720, can effectively upregulate SIRT1 expression and inhibit ROS production, thereby reversing PM-induced cell damage. This study provides a new potential target for clinical treatment of PM-associated ocular surface diseases.

Keywords: particulate matter, corneal/conjunctival epithelial cells, oxidative stress, reactive oxygen species, silent information regulator 1, SRT1720

In recent decades, air pollution has become a global mainstream environmental pollution and public health issue.[1] Particulate matter (PM) is the main factor leading to the deterioration of air quality, and it is also the most harmful pollutant in the atmospheric environment. Therefore its toxicological mechanism and health hazards have become a hot issue of concern.[2,3] The human body can be exposed to PM through all possible routes, including inhalation, ingestion, skin contact, and ocular surface contact, thus widely affecting human health.[4,5] The ocular surface as a highly susceptible ocular tissue to PM exposure has become an important target tissue for research on the effects of PM on eye health.[6,7]

At present, numerous studies have shown that the main mechanism of PM-induced ocular surface damage is related to its induced cytotoxicity, oxidative stress, and inflammatory response.[8,9] However, its toxicological mechanism and health hazards have not been fully elucidated. Nanotoxicology has been recognized as one of the important disciplines that contribute to the understanding of PM toxicological mechanisms due to the large similarities of toxicological mechanisms between PM and nanoparticles.[5,10–12] Recently, an in vitro study reported that carbon black and zinc oxide nanoparticles could induce oxidative stress damage and downregulated intracellular silent information regulator 1(SIRT1) protein expression, when they were exposed to human corneal epithelial cells (HCECs) and human conjunctival epithelial cells (HCjECs).[13] SIRT1 as a gene that can regulate aging, inflammation, and oxidative stress is a member of the most studied mammalian sirtuin

family that has attracted increasing attention as a hot therapeutic target for oxidative stress–associated diseases.[14–16]

It is worth mentioning that SIRT1 is widely found in eye tissues such as cornea, conjunctiva, iris, ciliary body, lens, and retina.[17] However, the research on ocular surface diseases is mainly focused on diabetes-related corneal diseases.[18–21] Many studies have confirmed that SIRT1 can be used as an effective target for the prevention and treatment of diabetes-related corneal diseases, which has attracted widespread attention.[18–21] On the other hand, Lai et al.[22] recently have demonstrated that SIRT1 is involved in the pathogenesis of PM-induced airway inflammation, and activation of SIRT1 could prevent airway disorders or disease exacerbations induced by airborne particulate pollution. However, to our knowledge, no studies to date have reported on the role of SIRT1 in PM-induced ocular surface damage.

Thus to explore whether SIRT1 is involved in the occurrence and development of PM-induced ocular surface damage, this study evaluated the effect of PM exposure on the expression of SIRT1 protein in HCECs and HCjECs. In addition, to explore whether the activation or upregulation of SIRT1 can exert a protective effect on PM-induced oxidative stress cell damage, this study conducted an intervention study using a SIRT1 activator (SRT1720, a selective SIRT1 synthetic activator). This study aims to explore a new potential therapeutic target for the prevention and clinical treatment of PM-associated ocular surface diseases.

Material and Methods

Reagents

Keratinocyte-serum free medium (K-SFM), keratinocyte supplements containing human recombinant epidermal growth factor and bovine pituitary extract, phenol red–free Dulbecco's modified Eagle medium, 0.05% trypsin–ethylenediaminetetraacetic acid, fetal bovine serum, 1 × phosphate-buffered saline solution (PBS), penicillin-streptomycin (10,000 U/mL), and radioimmunoprecipitation assay buffer (no. 89901) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Roswell Park Memorial Institute 1640 medium and bovine serum albumin (BSA) were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA). Fibronectin was purchased from Corning (Corning, NY, USA). Rat tail collagen type I was obtained from Advanced BioMatrix (San Diego, CA, USA). Water-soluble tetrazolium salt (WST)-8 cell viability kit was purchased from DoGen Bio (Seoul, Korea). SRT1720 (no. 1001645-58-4), a selective SIRT1 activator, was purchased from Selleckchem (Houston, TX, USA). Anti-SIRT1 (no. 8469) and β-actin (no. 5125) antibodies and senescence β-galactosidase (SA-β-gal) staining kit (no. 9860) were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-mouse immunoglobulin G horseradish peroxidase–linked antibody (no. PI-2000) was obtained from Vector Laboratories (Burlingham, NY, USA). The 2′,7′-dichlorofluorescein diacetate (DCFH-DA) cellular ROS detection assay kit (no. ab113851) and tetramethylrhodamine ethyl ester (TMRE) mitochondrial membrane potential (MMP) assay kit (no. ab113852) were purchased from Abcam (Cambridge, England). A fluorescein isothiocyanate (FITC) Annexin V apoptosis detection kit was obtained from BD Biosciences (San Jose, CA, USA). Finally, 96-well black plates, and six-well, 12-well, and 96-well cell culture plates were obtained from SPL Life Sciences (Pocheon, Korea).

Preparation of PM Suspension

An ambient PM sample (Standard Reference Material 1648a) was purchased from the National Institute of Standards and Technology (NIST), USA. This Standard Reference Material is atmospheric PM collected in an urban area.[23] The PM sample was prepared to PM suspension (dissolved in PBS), as previously described.[13] Briefly, PM sample was weighted on a high-precision microbalance, and a stock suspension was performed in sterile PBS at a concentration of 10 mg/mL. Before dilution in cell culture media, these suspensions were sonicated 40 minutes intermittently through a bath sonicator, then additionally sonicated 1 minute before their experimental use to minimize their aggregation.

Cell Culture and Treatment

HCECs (2.040 pRSV-T, CRL-11516) and HCjECs (clone 1-5c-4 [Wong-Kilbourne derivative of Chang conjunctiva], Korean Cell Line Bank no. 30052) were obtained from the American Type Culture Collection (Manassas, VA, USA) and Korean Cell Line Bank (Seoul, Korea), respectively.[13] The HCECs were maintained in K-SFM supplemented with 0.05 mg/mL of bovine pituitary extract, 5 ng/mL of epidermal growth factor, 100 U/mL of penicillin, and 100 µg/mL of streptomycin at 37°C in a 5% CO_2 incubator. The HCjECs were maintained in Roswell Park Memorial Institute 1640 medium containing 10% fetal bovine serum, 100 U/mL of penicillin, and 100 µg/mL of streptomycin at 37°C in a 5% CO_2 incubator.

For experiments, HCECs (2×10^5 cells/mL) and HCjECs (2×10^5 cells/mL) were seeded in 96-well, 12-well, and six-well plates with 100 µL, 1000 µL, and 2500 µL of culture medium, respectively.[13] Cells were incubated for 24 hours to reach 90% confluency and treated with PM at the indicated concentration. In addition, to evaluate the protective effects of SIRT1 activator on PM-induced cell injury to HCECs and HCjECs, the optimal working concentrations of SRT1720 were screened first by using cell viability assay. The cell viability was examined after treatment, and the results were compared with those of untreated cells (control group). Then, PM treated cells were co-incubated with or without SRT1720 under the screened optimal working concentrations for evaluation effect of SIRT1 activator.

Cell Viability Assay

Cytotoxicity was evaluated using a WST-8 cell viability assay kit.[13] Cells grown on 96-well plates were incubated with or without different concentrations of PM (100, 200, 400, 600, 800, or 1000 µg/mL) for 24 hours. In addition, to evaluate the safe concentration range of SRT1720 on cells, cells were incubated with or without different concentrations of SRT1720 (1.25 µM, 2.5 µM, or 5 µM) for 24 hours. The WST-8 solution (DoGen Bio, Seoul, Korea) was added to each well and cells were incubated for two hours; then, the colored supernatants were measured at 450 nm. The results are presented as percentages relative to the untreated control. The concentration of PM at which 50% of the cells were nonviable is known as the IC50, and its value was determined by nonlinear regression curve fitting using Graph-

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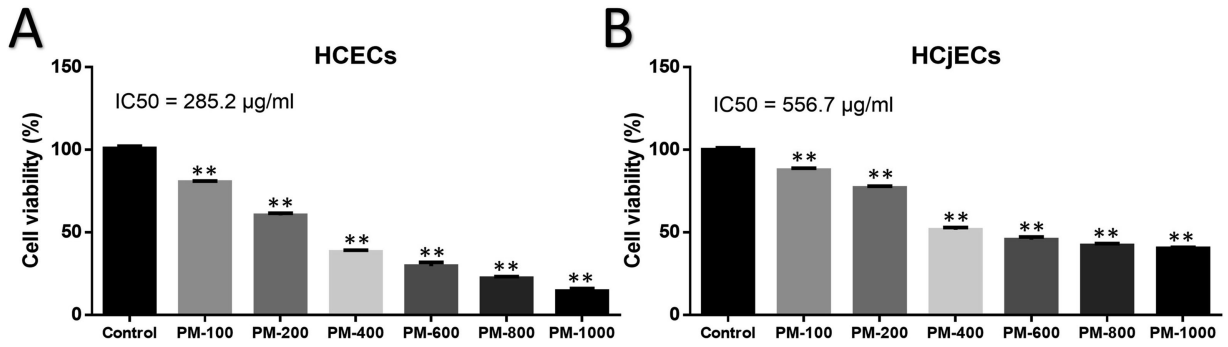

Figure 1. Cytotoxicity evaluation of PM with the WST-8 assay. HCECs (**A**) and HCjECs (**B**) were treated with different concentrations (100, 200, 400, 600, 800, or 1000 μg/mL) of PM for 24 hours. The IC50 values of PM are shown at the top left of **A** and **B**. Results are given in percentages related to untreated control cells. Results are shown as the mean ± standard deviation values of three independent experiments, each of which was carried out in triplicate. $^{*}P < 0.05$ and $^{**}P < 0.01$, versus untreated controls.

Pad Prism 5.0 software.[13] All experiments were performed in triplicate.

Based on cytotoxicity test results (as shown in Fig. 1 and Fig. 2), this study selected 200 μg/mL and 2.5 μM as the optimal working concentrations of PM and SRT1720 for the experiments, respectively.

Protein Extraction and Western Blotting

Cells were seeded in six-well plates and cultured overnight to allow for attachment. After being treated with 200 μg/mL PM and 2.5 μM SRT1720 for 24 hours, cells were lysed in radio-immunoprecipitation assay buffer for 30 minutes on ice. Protein concentrations were determined using the bicinchoninic acid method. Proteins were separated by 8% polyacrylamide gel electrophoresis containing 0.1% sodium dodecyl sulfate and transferred to polyvinylidene difluoride membranes. The nitrocellulose membrane was blocked with 5% BSA and subsequently incubated with SIRT1 monoclonal antibody (1:1000 dilution) overnight at 4°C. Then, the membranes were incubated with horseradish peroxidase–conjugated horse anti-mouse immunoglobulin G secondary antibody (1:2000 dilution) for another hour. The β-actin (1:10,000 dilution) was used as the loading control, and the ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to calculate protein intensity. The results of Western blot analysis were expressed as a ratio to β-actin, as previously described.[24–26]

Intracellular Generation of ROS

DCFH-DA was used to measure intracellular ROS using the DCFH-DA cellular ROS detection assay kit according to the manufacturer's protocol. Because DCFDH-DA oxidation assay was used to quantitatively assess ROS in live cell samples, we measured the ROS generation at an earlier time point (three hours) after exposure to PM, as previously described.[13,27] Cells grown on 12-well plates were treated with 200 μg/mL PM and 2.5 μM SRT1720 for three hours. Diluted DCFH-DA (10 μM) was then added to the cells, which were subsequently incubated for 30 minutes in the dark. A total of 10,000 events (cells) were analyzed by flow cytometry using the LSRFortessa X-20 program (BD Biosciences) at excitation/emission wavelengths of 488/535 nm. The results were expressed as percentages of increase over control cells (as 100%), as previously described.[13]

Cell Apoptosis Assay

Apoptosis was analyzed by an Annexin V-FITC/propidium iodide (PI) dual staining kit. Cells grown on six-well plates were treated with 200 μg/mL PM and 2.5 μM SRT1720 for 24 hours. According to the manufacture's instruction, the collected cells were resuspended in binding buffer and stained with 10 μL Annexin V-FITC and PI for 15 minutes in the dark. A total of 10,000 events (cells) were analyzed by flow cytometry using the LSRFortessa X-20 program (BD Biosciences). Four quadrants (Q1, Q2, Q3, and Q4) represented dead cells, late apoptotic cells, normal cells, and early apoptotic cells, respectively, in flow cytometry results. The percentage of total apoptotic cell was calculated using 100 × (early [Q4] + late [Q2]) apoptotic cell number/total cell number, as previously described.[28]

Detection of MMP

Cells were seeded in 96-well black plates and cultured overnight to allow for attachment. After treated with 200 μg/mL PM and 2.5 μM SRT1720 for 24 hours, the MMP was assessed using the TMRE assay. Cells were incubated for 30 minutes with TMRE at 500 nM diluted in warm culture medium at 37°C and 5% CO_2. Cells were then washed twice with warm PBS/0.2% BSA and fluorescence intensity was detected using a fluorescence plate reader with excitation/emission wavelengths at 549/575 nm, as previously described.[13]

Cell Senescence Assay

Cellular senescence was analyzed by SA-β-gal staining kit. Cells grown on 12-well plates were treated with 200 μg/mL PM and 2.5 μM SRT1720 for 24 hours. According to the manufacture's instruction, cells were fixed with 1× fixative solution for 15 min and then stained overnight at 37°C with the β-galactosidase staining solution at pH 6.0 for 15 hours. Cell staining was observed under a light microscope at magnification × 200, and digital images were acquired by Olympus BX51 microscope and a DP72 camera (Olympus Optical Co., Ltd., Tokyo, Japan). Areas measuring 0.26 mm × 0.26 mm (length × width) from each image were scanned. We selected type 8-bit, used the "adjust," "threshold max 180," and "measure" commands in ImageJ. The results

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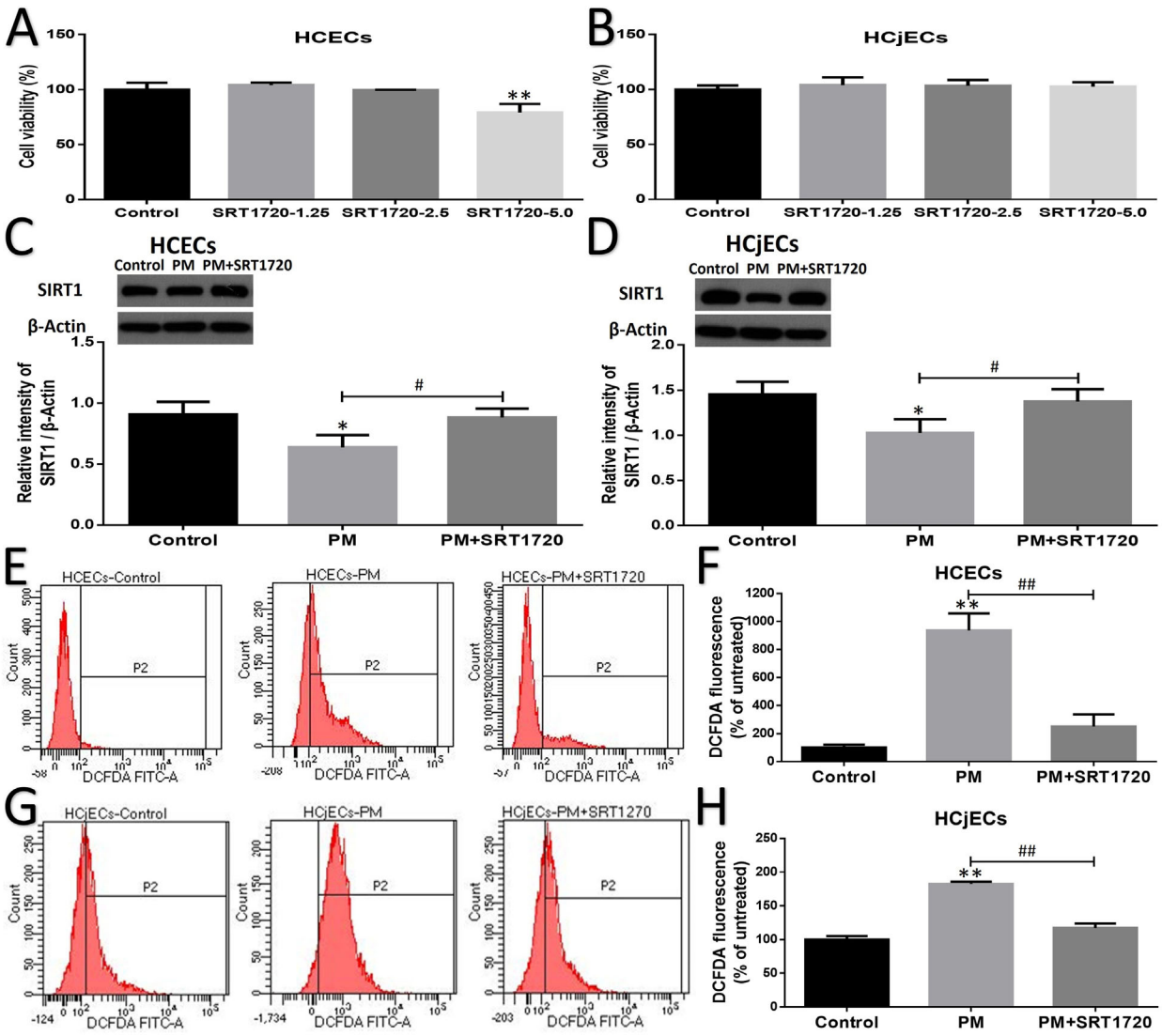

Figure 2. Cell viability of HCECs (**A**) and HCjECs (**B**) was determined by the WST-8 assay after treatment with different concentrations (1.25 µM, 2.5 µM, and 5 µM) of SRT1720 for 24 hours. Results are given in percentages related to untreated control cells. SIRT1 expression of HCECs (**C**) and HCjECs (**D**) was determined by the Western blot analyses after treatment with 200 µg/mL of PM and 2.5 µM of SRT1720 for 24 hours. Results are expressed as a ratio to β-actin. Intracellular ROS generation of HCECs (**E**, **F**) and HCjECs (**G**, **H**) was determined by the DCFDH-DA oxidation assay after treatment with 200 µg/mL of PM and 2.5 µM of SRT1720 for three hours. The representative figures are shown, and DCFDA-positive cells (*P2*) were calculated as a percentage of the controls. All results are shown as the mean ± standard deviation values of three independent experiments, each of which was carried out in triplicate. $^{*}P < 0.05$ and $^{**}P < 0.01$, versus untreated control. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, PM+SRT1720 group versus PM group.

of "% area" were used as the stained level of SA-β-gal, as previously described.[29–31]

Statistical Analyses

All data were expressed as the mean ± standard deviation values of at least three independent experiments. Comparison between two groups (control vs. PM or PM + SRT1720 vs. PM) for statistical difference were performed with Student's two-tailed *t*-test. One-way ANOVA was used to determine statistical significance for multiple groups analysis. All statistical analyses were performed using the Statistical Package for Social Sciences version 20.0 program (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

Results

Dose- and Cell Type-Dependent Impacts of PM on Cytotoxicity in Cultured Human Ocular Surface Epithelial Cells

To determine the cytotoxicity of PM on HCECs and HCjECs, we evaluated the cell viability by WST assay. A dose-dependent decrease in cell viability was observed when the HCECs and HCjECs were exposed to different doses of PM for 24 hours (Fig. 1). In addition, the HCEC cell line (2.040 pRSV-T) seems to be more susceptible to PM toxicological effects than HCjECs cell line (Wong-Kilbourne derivative of Chang conjunctiva) in this study. Our data showed that the IC50 of PM in HCECs and HCjECs are 285.2 µg/ml and

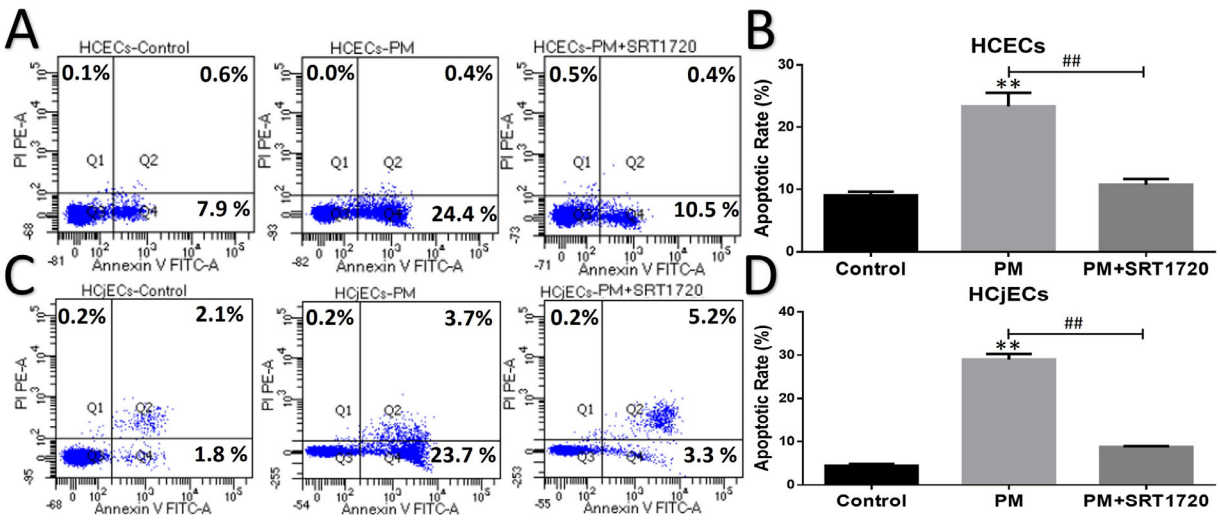

Figure 3. Cellular apoptosis was detected by annexin V apoptosis assay. HCECs (**A, B**) and HCjECs (**C, D**) were treated with 200 µg/mL of PM and 2.5 µM of SRT1720 for 24 hours. The percentage of total apoptotic cell was calculated using 100 × (early [Q4] + late [Q2]) apoptotic cell number/total cell number. Results are shown as the mean ± standard deviation values of three independent experiments, each of which was carried out in triplicate. *$P < 0.05$ and **$P < 0.01$, PM group versus untreated control. #$P < 0.05$ and ##$P < 0.01$, PM+SRT1720 group versus PM group.

556.7 µg/ml, respectively, as shown in Figure 1. The IC50 of PM in HCjECs was about 1.95 times higher than that in HCECs. These results suggest that PM-induced ocular surface epithelial cytotoxicity is dependent on dose and cell type.

To further analyze cellular responses induced by PM exposure in HCECs and HCjECs, we chose a concentration of 200 µg/mL (based on IC50 of PM) as the PM exposure dose for the following studies, the dose of which can cause cell viability reduction in HCECs and HCjECs as 40% ± 1.3% and 23% ± 1.1%, respectively.

SIRT1 Activator SRT1720 Inhibits ROS Formation Induced by PM Exposure

To determine whether SIRT1 expression changed by PM exposure in HCECs and HCjECs and explore whether the activation or upregulation of SIRT1 can exert a protective effect on PM-induced cell damage. On the other hand, because previous studies have shown that high concentrations of SRT1720 may induce cytotoxicity,[32] we first tested the cytotoxicity of HCECs and HCjECs exposed to multiple concentrations of SRT1720 for 24 hours, and only 5 µM SRT1720 provoked a significant reduction in HCECs viability ($P = 0.048$; Fig. 2A and Supplementary Table S1). Therefore we used a relatively low concentration (2.5 µM) of SRT1720 for the following studies.

ROS has been suggested to be an important biomarker in the evaluation for toxicological effects of PM.[7,33,34] It has been reported that the overproduction of ROS is negatively associated with the expression of SIRT1 that in various oxidative stress conditions.[35–37] Our results found that PM exposure significantly inhibited SIRT1 expression and increased DCF fluorescence intensity, when compared with the control group in HCECs ($P = 0.014$ and $P = 0.00004$, respectively; Figs. 2C, 2E, 2F) and HCjECs ($P = 0.015$ and $P = 0.00004$, respectively; Figs. 2D, 2G, 2H). In contrast, SRT1720 treatment significantly upregulated SIRT1 expression and decreased DCF fluorescence intensity, when compared with the PM-exposed group in HCECs ($P = 0.0047$ and $P = 0.0002$, respectively; Figs. 2C, 2E, 2F) and HCjECs ($P = 0.034$ and $P = 0.0001$, respectively; Figs. 2D, 2G, 2H). These results suggest that the expression of SIRT1 is significantly downregulated during PM-induced cellular oxidative stress injury, which may be related to the overproduction of ROS. Fortunately, this progress can be effectively reversed by the application of SIRT1 activator SRT1720.

SIRT1 Activator SRT1720 Ameliorates Apoptosis Induced by PM Exposure

Excessive intracellular ROS can cause damage to proteins, nucleic acids, lipids, membranes, and organelles, leading to apoptosis.[38] Detection and quantification of apoptosis were carried out by annexin V apoptosis assay. Our results found that PM exposure significantly increased apoptosis when compared with controls in HCECs ($P = 0.0004$; Figs. 3A, 3B) and HCjECs ($P = 0.00001$; Figs. 3C, 3D). In contrast, SRT1720 treatment significantly inhibited PM-induced apoptosis when compared with the PM-exposed group in HCECs ($P = 0.0008$; Figs. 3A, 3B) and HCjECs ($P = 0.001$; Figs. 3C, 3D). These results suggest that PM exposure can induce ROS-mediated apoptosis but can be effectively reversed by SIRT1 activator, which seems to be associated with its inhibitory effect to ROS production.

SIRT1 Activator SRT1720 Ameliorates Mitochondrial Damage Induced by PM Exposure

Mitochondria are a major source of intracellular ROS, which are particularly vulnerable to oxidative stress and can lead to its damage.[39,40] MMP was detected using the TMRE assay for evaluated mitochondrial function. Our results found that PM exposure significantly decreased TMRE fluorescence intensity when compared with controls in HCECs

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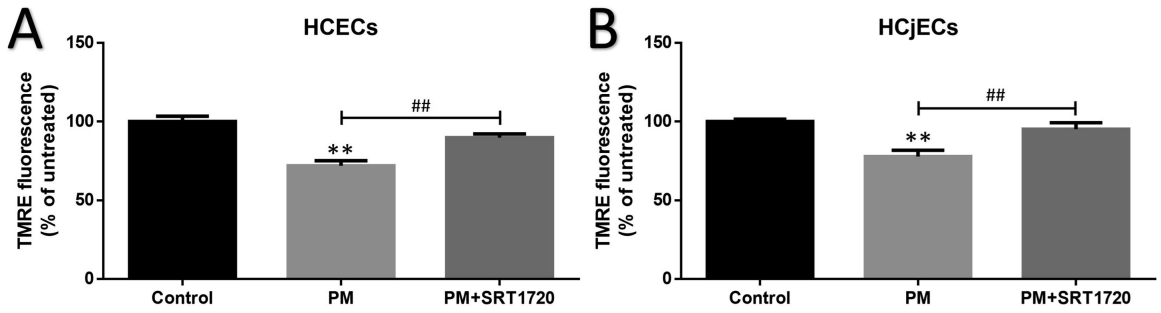

Figure 4. Mitochondrial membrane potential was measured by using the TMRE assay. HCECs (**A**) and HCjECs (**B**) were treated with 200 µg/mL of PM and 2.5 µM of SRT1720 for 24 hours. The results were calculated as a percentage of TMRE fluorescence intensity relative to controls. Results are shown as the mean ± standard deviation values of three independent experiments, each of which was carried out in triplicate. $^{*}P < 0.05$ and $^{**}P < 0.01$, PM group versus untreated control. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, PM+SRT1720 group versus PM group.

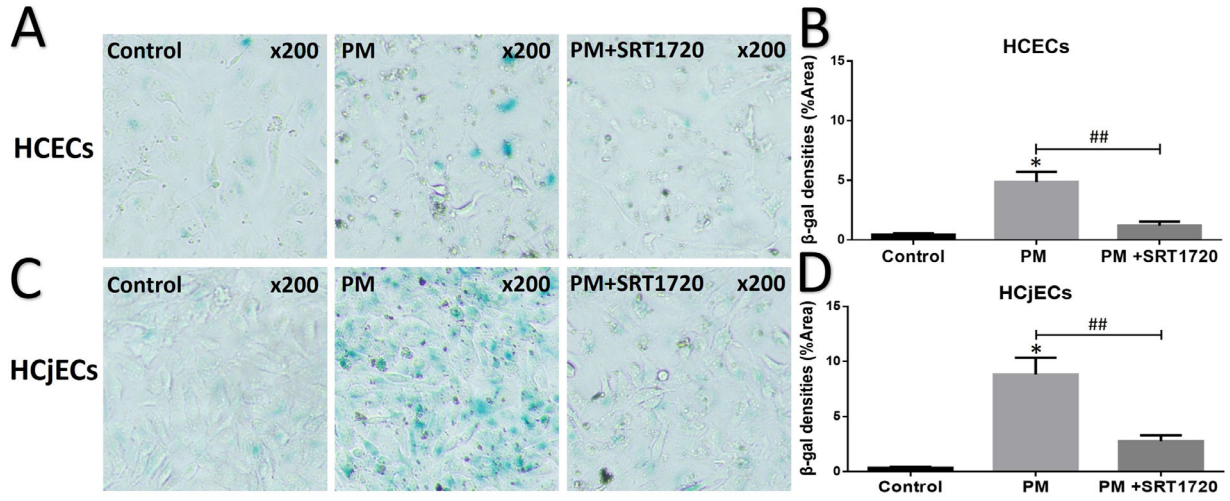

Figure 5. Cellular senescence was evaluated by SA-β-gal staining. Representative SA-β-gal staining images of HCECs (**A**) and HCjECs (**C**) treated with 200 µg/mL of PM and 2.5 µM of SRT1720 for 24 hours. (**B**) Stained levels of SA-β-gal–positive cells in HCECs. (**D**) Stained levels of SA-β-gal-positive cells in HCjECs. The results of "% area" were used as the stained level of SA-β-gal, digitized for analysis by ImageJ software. Results are shown as the mean ± standard deviation values of three independent experiments, each of which was carried out in triplicate. $^{*}P < 0.05$ and $^{**}P < 0.01$, PM group versus untreated control. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, PM+SRT1720 group versus PM group.

($P = 0.0005$; Fig. 4A) and HCjECs ($P = 0.0009$; Fig. 4B). In contrast, SRT1720 treatment significantly increased PM-induced TMRE fluorescence intensity when compared with the PM-exposed group in HCECs ($P = 0.0016$; Fig. 4A) and HCjECs ($P = 0.0064$; Fig. 4B). These results suggest that PM exposure can induce ROS-mediated mitochondrial damage but can be effectively reversed by SIRT1 activator, which seems to be associated with its inhibitory effect to ROS production.

SIRT1 Activator SRT1720 Ameliorates Cell Senescence Induced by PM Exposure

At the cellular level, increased ROS leads to cellular senescence among other cellular fates including apoptosis, necrosis, and autophagy.[41,42] Cellular senescence was assessed using the SA-β-gal staining. Our results found that PM exposure significantly increased the levels of SA-β-gal-positive cell staining when compared with controls in HCECs ($P = 0.0109$; Figs. 5A, 5B) and HCjECs ($P = 0.0105$; Figs. 5C, 5D). In contrast, SRT1720 treatment significantly inhibited PM-induced the levels of SA-β-gal-positive cell staining when compared with the PM-exposed group in HCECs ($P = 0.0023$; Figs. 5A, 5B) and HCjECs ($P = 0.0029$; Figs. 5C, 5D). These results suggest that PM exposure can induce ROS-mediated cell senescence but can be effectively reversed by SIRT1 activator, which seems to be associated with its inhibitory effect to ROS production.

SIRT1 Activator SRT1720 Reverses Cytotoxicity Induced by PM Exposure

Finally, we verified whether SRT1720 could improve PM-induced cytotoxicity in HCECs and HCjECs. As expected, SRT1720 treatment significantly increased cell viability when compared with the PM-exposed group in HCECs ($P = 0.0016$; Fig. 6A) and HCjECs ($P = 0.0064$; Fig. 6B). These results suggest that SIRT1 activator SRT1720 reverses PM-induced cytotoxicity by inhibiting ROS production and oxidative stress–mediated cellular damage.

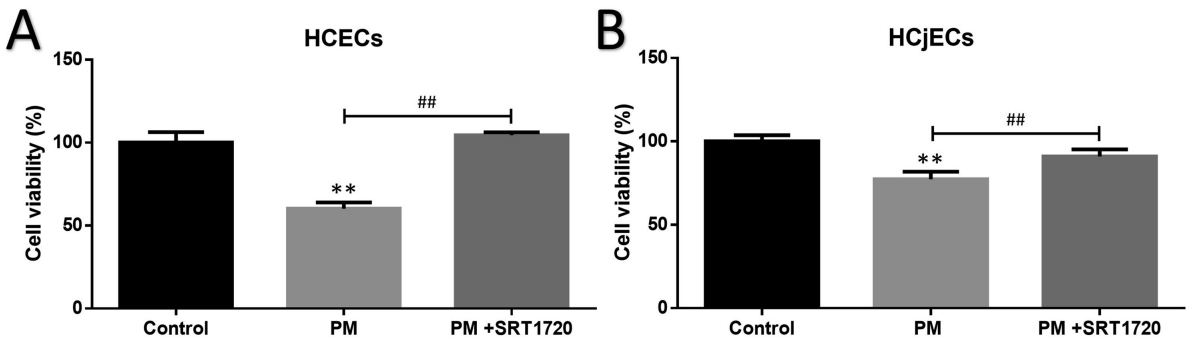

Figure 6. Effect of SIRT1 activator SRT1720 on PM induced cytotoxicity was evaluated by WST-8 assay. HCECs (**A**) and HCjECs (**B**) were treated with 200 µg/mL of PM and 2.5 µM of SRT1720 for 24 hours. Results are shown as the mean ± standard deviation values of three independent experiments, each of which was carried out in triplicate. *$P < 0.05$ and **$P < 0.01$, PM group versus untreated control. #$P < 0.05$ and ##$P < 0.01$, PM+SRT1720 group versus PM group.

Discussion

PM is one of the important environmental factors that affect ocular surface health.[6] Numerous epidemiological and clinical studies have shown that PM exposure is positively correlated with outpatient visits for various ocular surface diseases, including conjunctivitis, blepharitis, pterygium, and dry eye.[43–47] It is reported that PM exposure can induce a range of ocular symptoms, including irritation, foreign body sensation, burning sensation, itching, photophobia, and lacrimation.[48,49] In addition, it can lead to various ocular surface dysfunctions, including decreased tear film stability, increased secretion of meibomian glands, and hyperplasia of conjunctival goblet cells.[46,49–51] On the other hand, PM exposure can induce ocular surface diseases, including dry eye and allergic conjunctivitis in experimental animals.[52–55] At present, numerous studies have confirmed the mechanism of PM-induced ocular surface damage, including inflammatory response, oxidative stress, cytotoxicity, DNA damage, and cell senescence.[8,9,27,33,56] Therefore the corresponding prevention and intervention treatment will be a very urgent scientific issue and a new research hotspot.

SIRT1 is a nicotinamide-adenine dinucleotide–dependent histone/non-histone deacetylase, a gene that can regulate glucose and lipid metabolism, inflammatory response, cell senescence, cell apoptosis, and oxidative stress.[57,58] Because SIRT1 plays an important role in maintaining the healthy state of the body, it has been widely studied as a therapeutic target for various diseases.[57,58] It has been reported that SIRT1 can inhibit ROS production by regulating many downstream target proteins (such as p53, peroxisome proliferator–activated receptor-gamma coactivator-1 alpha [PGC-1α], nuclear factor kappa-B, and class O of forkhead box.), thereby exerting an antioxidant effect.[59–65] However, it is worth mentioning that previous studies have suggested that there is a complex interaction and optimal balance between SIRT1 and ROS to maintain physiological homeostasis, although the signaling crosstalk between them has not been fully elucidated.[66] Numerous studies have reported that oxidative stress conditions (i.e., ROS overproduction) can lead to downregulation of SIRT1 expression by inducing overexpression of miRNA targets upstream of SIRT1.[35–37] In the present study we found that PM exposure induces massive production of ROS that mediated cellular oxidative stress in HCECs and HCjECs, including apoptosis, mitochondrial damage, and cellular senescence. In addition, the expression of SIRT1 in these two cells were also downregulated by PM exposure, which seems to be related to the overproduction of ROS leading to a dysregulation of the balance between ROS and SIRT1, thereby eliminating or attenuating its antioxidant effect on PM exposure.

Previous studies have reported that SIRT1 activators (such as SRT1720, resveratrol, melatonin, etc.) activate its antioxidant-related pathways (such as p53, PGC-1α, nuclear factor kappa-B, class O of forkhead box,) by activating or upregulating its expression, thereby playing a protective role in many oxidative stress- associated diseases.[67–73] Lai et al.[22] demonstrated that the SIRT1 activator resveratrol (100 mg/kg) injected intraperitoneally before PM challenge significantly increased the SIRT1 in mouse lungs and inhibited PM-induced airway inflammation in mice (such as IL-6 and IL-8 cytokine production). In addition, Liang et al.[70] demonstrated that the SIRT1 activator SRT1720 could prevent oxidative stress-mediated ROS production in intestinal epithelial cells through activation of the SIRT1/PGC-1α pathway. It is reported that the half-life of SRT1720 is longer than that of resveratrol, and its affinity with SIRT1 is about 1000 times that of resveratrol.[73] Therefore SRT1720 was selected to explore the protective effect of a SIRT1 activator on PM-induced corneal and conjunctival epithelial cell damage. As shown in our results, this study found that SRT1720 treatment could effectively reverse the PM-induced downregulation of SIRT1 expression and keep the level in control. Meanwhile, SRT1720 treatment significantly inhibited ROS production, thereby reversing PM-induced cellular oxidative stress and cytotoxicity. These findings suggest that SRT1720, as a SIRT1 activator, can play a protective role in PM-induced oxidative stress injury in HCECs and HCjECs, which provides a new idea and theoretical basis for the prevention and clinical treatment of PM-induced ocular surface injury.

However, there are some shortcomings in this study. To better understand the role of SIRT1 in PM-induced cell damage, further studies on its related target proteins are needed to clarify its potential regulatory mechanism. In addition, efficacy of SRT1720 at the in vivo level remains to be determined in further studies. It is worth mentioning that previous studies have shown that oral or intraperitoneal injection of high doses of SRT1720 in experimental animals can lead to plasma glucose reduction and tissue fibrosis, as well as intolerance (such as decreased weight gain and food intake and even death),[73–76] but these effects did not

occur at low doses.[75,77,78] Therefore choosing a relatively low concentration of SRT1720 as a therapeutic dose for PM-induced ocular surface damage in future research may be an ideal solution to avoid potential undesirable effects. On the other hand, further investigation of potential differences between acute exposure to PM and real-life chronic exposure to PM will lead to a better understanding of the impact of PM on ocular surface health. A previous study showed that short-term exposure to PM was positively associated with lower tear film break-up time, but the association was lost with long-term PM exposure.[49] Interestingly, long-term exposure to PM appears to be positively associated with increased tear volume, as well as increased ocular surface mucin and lipid secretion.[46,49,79] Finally, we suggest that the main limitation of this in vitro study is the inability to mimic these ocular surface defense mechanisms (e.g., mucin and lipid secretion) that may present during in vivo (real-life) PM exposure. Therefore, in addition to the toxicological mechanisms of PM, in-depth exploration of the differential effects of short- and long-term PM exposure on the defense mechanisms of the ocular surface in vivo is crucial to elucidate the potential differences between acute and chronic exposure of PM on the ocular surface.

Overall, although SIRT1 has been shown to be a potential therapeutic target in various oxidative stress–associated diseases, its research focus in ocular surface diseases is limited to diabetes-related corneal diseases. This study is the first to find that SIRT1 is involved in the occurrence and development of PM exposure-induced ocular surface epithelial cell damage. PM exposure induced ROS-mediated oxidative stress cell damage in HCECs and HCjECs, while inhibiting the expression of SIRT1, thereby attenuating its antioxidant effect to PM exposure. However, SIRT1 activator SRT1720 can effectively solve this problem by upregulating its expression so that it can fully exert its antioxidant effect to PM exposure. These findings may provide valuable insights for the future treatment of PM-associated ocular surface diseases.

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Investigative Ophthalmology & Visual Science

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