



Pharmacological inhibition of PAI-1 alleviates cardiopulmonary pathologies induced by exposure to air pollutants PM_{2.5}[☆]

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ABSTRACT

Numerous studies have established that acute or chronic exposure to environmental pollutants like particulate matter (PM) leads to the development of accelerated aging related pathologies including pulmonary and cardiovascular diseases, and thus air pollution is one of the major global threats to human health. Air pollutant particulate matter 2.5 (PM_{2.5})-induced cellular dysfunction impairs tissue homeostasis and causes vascular and cardiopulmonary damage. To test a hypothesis that elevated plasminogen activator inhibitor-1 (PAI-1) levels play a pivotal role in air pollutant-induced cardiopulmonary pathologies, we examined the efficacy of a drug-like novel inhibitor of PAI-1, TM5614, in treating PM_{2.5}-induced vascular and cardiopulmonary pathologies. Results from biochemical, histological, and immunohistochemical studies revealed that PM_{2.5} increases the circulating levels of PAI-1 and thrombin and that TM5614 treatment completely abrogates these effects in plasma. PM_{2.5} significantly augments the levels of pro-inflammatory cytokine interleukin-6 (IL-6) in bronchoalveolar lavage fluid (BALF), and this also can be reversed by TM5614, indicating its efficacy in amelioration of PM_{2.5}-induced increases in inflammatory and pro-thrombotic factors. TM5614 reduces PM_{2.5}-induced increased levels of inflammatory markers cluster of differentiation 107 b (Mac3) and phospho-signal transducer and activator of transcription-3 (pSTAT3), adhesion molecule vascular cell adhesion molecule 1 (VCAM1), and apoptotic marker cleaved caspase 3. Longer exposure to PM_{2.5} induces pulmonary and cardiac thrombosis, but TM5614 significantly ameliorates PM_{2.5}-induced vascular thrombosis. TM5614 also reduces PM_{2.5}-induced increased blood pressure and heart weight. In vitro cell culture studies revealed that PM_{2.5} induces the levels of PAI-1, type I collagen, fibronectin (Millipore), and sterol regulatory element binding protein-1 and 2 (SREBP-1 and SREBP-2), transcription factors that mediate profibrogenic signaling, in cardiac fibroblasts. TM5614 abrogated that stimulation, indicating that it may block PM_{2.5}-induced PAI-1 and profibrogenic signaling through suppression of SREBP-1 and 2. Furthermore, TM5614 blocked PM_{2.5}-mediated suppression of nuclear factor erythroid related factor 2 (Nrf2), a major antioxidant regulator, in cardiac fibroblasts. Pharmacological inhibition of PAI-1 with TM5614 is a promising therapeutic approach to control air pollutant PM_{2.5}-induced cardiopulmonary and vascular pathologies.

1. Introduction

Air pollution-associated toxicity is a major threat to human health. Exposure to air pollutants leads to development of pulmonary and cardiovascular disease-related morbidity and mortality. Environmental pollutants like ozone, nitrogen oxide, and particulate matter (PM)

induce oxidative stress, which leads to cardiopulmonary vascular diseases (CPVDs) including thrombosis, hypertension, arteriosclerosis, arrhythmias, and myocardial infarction—affecting millions of people worldwide (Du et al., 2016; Bourdrel et al., 2017). PM is the most dangerous environmental pollutant, as these solid particles contain a wide variety of toxic substances including sulfates, nitrates,

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hydrocarbons, heavy metals, and bacteria. Based on its aerodynamic diameter, PM has been classified as coarse (PM₁₀), fine (PM_{2.5}), and ultrafine (PM_{0.1}) (Brook et al., 2004, 2010). The detrimental effects of PM on the cardiopulmonary/vascular system stem from chronic or acute exposure-induced oxidative stress and systemic inflammation, cellular dysfunction, and impaired tissue homeostasis (Brook et al., 2004, 2010; Nel, 2005; Simkhovich et al., 2009; Franchini and Mannucci, 2011; Budinger and Mutlu, 2011; Du et al., 2016; Bourdrel et al., 2017). While the acute effects of PM may be direct, involving rapid crossover from the lung epithelium into the circulation, the chronic effects of PM involve generation of pulmonary oxidative stress, systemic inflammation, and cellular dysfunction (Nelin et al., 2012; Robertson and Miller, 2018; Wu et al., 2019). With time, both direct and indirect effects of PM facilitate the onset and progression of CPVD pathologies. However, the exact molecular mechanism by which environmental pollutants like PM_{2.5} contribute to cardiopulmonary vascular diseases is not fully understood.

Plasminogen activator inhibitor-1 (PAI-1), a secretory protein that inhibits t-PA/uPA serine proteases, regulates the tissue fibrinolytic system, and its deregulation contributes to different diseases, including CPVDs like thrombosis and accelerated cellular senescence and aging (Westrick and Eitzman, 2007; Ghosh and Vaughan, 2012; Ghosh et al., 2016; Vaughan et al., 2017; Sun et al., 2019). Here, we tested a hypothesis that elevation of PAI-1 plays a pivotal role in PM-induced CPVDs by inhibiting t-PA, increasing oxidative stress, and promoting cellular dysfunction and cardiopulmonary vascular injury; conversely, pharmacological inhibition of PAI-1 ameliorates PM-induced CPVDs. The strong rationale behind formulating this hypothesis comes from the previous reports that (i) the levels of PAI-1 are significantly elevated in oxidative stress-induced cardiac cells, (ii) elevated PAI-1 is a key determinant of premature cellular senescence and dysfunction in different pulmonary and cardiac cells, and (iii) pharmacological inhibition of PAI-1 effectively blocks oxidative stress-induced cellular dysfunction through suppression of key senescence mediators (Ghosh et al., 2016; Vaughan et al., 2017; Sun et al., 2019). In addition, the levels of PAI-1 are significantly elevated in bronchoalveolar lavage fluid (BALF) in mice, and in lung and heart tissues from rats, exposed to PM_{2.5} and are associated with inflammation and apoptosis (Upadhyay et al., 2010; Budinger et al., 2011). These findings strongly predict a therapeutically relevant function of PAI-1 inhibition in preventing PM_{2.5}-induced CPVDs. In this study, we tested the efficacy of a novel drug-like small molecule inhibitor of PAI-1, TM5614, in amelioration of air pollutant PM_{2.5}-induced CPVD pathologies. The N-acylanthranilic acid derivative TM5614 is designed based on the structure of original compounds TM5001 and TM5007. TM5614 is a novel PAI-1 inhibitor with better oral bioavailability that selectively inhibits PAI-1 activity (Yamaoka et al., 2018; Yahata et al., 2021). Recently, TM5614 received FDA approval for clinical trial to assess the efficacy and safety of TM5614 for high-risk patients with severe COVID-19 symptoms (Clinical trials.gov Identifier: NCT04634799). The results of the present investigation reveal that pharmacological inhibition of PAI-1 in mice is associated with suppression of PM_{2.5}-induced inflammatory and prothrombotic factors and thus of CPVD pathologies including vascular thrombosis.

2. Materials and Methods

All data and supporting materials have been provided with the published article and in the data supplement.

2.1. Animal studies

Wild-type, PAI-1 heterozygous, and PAI-1 knockout C57BL/6 mice (9–12 weeks old) were used for this study. All mouse protocols were approved by the Northwestern University Animal Care and Use Committee. Wild-type male mice were divided into 4 groups. Group 1: mice were fed with regular chow and received 50 µl of phosphate-buffered

saline (PBS as negative control) (25 µl, twice) by intratracheal instillation. Group 2: mice were fed with regular chow and received particulate matter 2.5 (PM_{2.5} was suspended in 50 µl of sterile PBS) (the National Institutes of Standards and Technology, Gaithersburg, MD, USA; NIST SRM 1649) (50 µg–200µg/mouse; 50 µg or 100 µg or 200 µg in 50 µl PBS; 25 µl, twice) by intratracheal instillation. Doses of PM_{2.5} were selected based on well-accepted published literature (Urich et al., 2009; Soberanes et al., 2009; Upadhyay et al., 2010; Budinger et al., 2011; Pei et al., 2016; Yang et al., 2019; Huang et al., 2019; Jeong et al., 2019). Group 3: mice were fed with chow containing TM5614 (10 mg/kg/day) (dose has been selected based on previous published reports using TM5614 molecule) (Yahata et al., 2021) for 6 days and then received PBS (25 µl, twice) and TM5614 chow. Group 4: mice were fed with chow containing TM5614 (10 mg/kg/day) for 6 days and then received PM_{2.5} (50 or 200 µg/mouse in 50 µl PBS; 25 µl, twice) and TM5614 chow. After 24 h or 72 h (short-term exposure to PM_{2.5}: 50 or 200 µg/mouse or PBS in the presence or absence of TM5614) or after 4 weeks (long-term exposure to PM_{2.5}: 100 µg/mouse/week or PBS in the presence or absence of TM5614), mice were sacrificed and plasma and BALF (short-term exposure only) were collected and processed for biochemical analysis. For PAI-1 genetic deficiency study, batches of wild-type, PAI-1 heterozygous, and PAI-1 knockout male and female mice were fed with regular chow and treated either with PM_{2.5} (50 µg/mouse/week for 4 weeks) or PBS as controls. Hearts and lungs from short-term exposed mice (72 h to study the early pathological events) and long-term-exposed mice (4 weeks to study late pathological events) were collected and used for histological and immunohistochemical analysis. In vivo experimental plan has been presented in Table 1 under A-D.

2.2. Measurement of PAI-1 and thrombin-antithrombin (TAT) complex levels in plasma

Plasma was collected from the 4 groups of wild-type mice, and 50 µl of TAT standard and each sample were used in duplicate for TAT assays using a mouse ELISA kit for TAT (cat no ab137994, Abcam). Similarly, PAI-1 standard and each plasma sample were used in duplicate for PAI-1 assays using a mouse PAI-1 ELISA kit (cat no ab197752, Abcam).

2.3. Measurement of IL-6 in BALF

BALF was collected from each of the 4 groups of mice after 24 h of treatment as indicated above, and 50 µl of IL-6 standard and each sample were used in duplicate for IL-6 protein measurement using a Quantikine IL-6 mouse ELISA kit (cat no M6000B, R&D).

2.4. Blood pressure and echocardiography

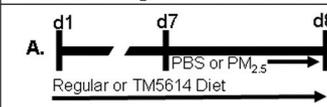
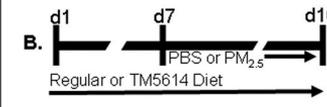
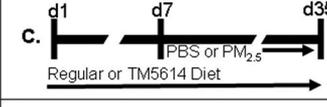
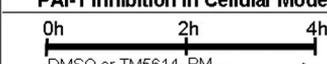
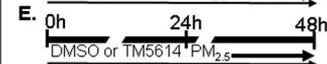
To monitor the effect of PM_{2.5} exposure on blood pressure, both systolic and diastolic blood pressure were measured in conscious mice using a non-invasive tail-cuff system (CODA Non-Invasive Blood Pressure, Kent Scientific Corp.). Trans-thoracic two-dimensional M-mode echocardiography was performed using a Vevo 3100 device (FujiFilm VisualSonics Inc., Toronto, Canada) equipped with a MX550D transducer. Echocardiographic studies were performed following manufacturer's instruction (<https://www.visualsonics.com/product/transducer/s/mx-series-transducers>) after 4 weeks of PM_{2.5} exposure. M-mode tracings were used to measure left ventricular (LV) wall thickness, LV dimensions, LV volume, and LV mass. Percent fractional shortening (% FS) and ejection fraction (% EF) were also determined. The mean value of at least 3–5 cardiac cycles was used to determine the measurements for each animal in all four groups. Post-mortem heart weight and body weight of each mouse in all four groups were recorded and compared.

2.5. Histology

Heart and lung tissues were collected and fixed in 10% formalin

Table 1

Experimental design, endpoint measurements and results of each experimental condition. Mice were fed with TM5614 chow or normal chow for 6 days followed by treatment with PBS or PM_{2.5} for 24 h (A) or 72 h (B) or 4 weeks (C) in the presence and absence of TM5614. Wildtype (PAI-1^{+/+}), heterozygous (PAI-1^{+/-}) and knockout (PAI-1^{-/-}) mice were treated with PBS or PM_{2.5} for 4 weeks (D). Effect of TM5614 on PM_{2.5}-induced cellular stress and abnormalities (E). Gr1: Group 1; Gr2: Group 2; Gr3: Group 3; Gr4: Group 4. Expression levels: +/+ > + > +/- > -.

Pharmacologic Inhibition of PAI-1	Endpoint Measurements	Results				Sections	
A. 	Plasma: PAI-1, Thrombin BAL Fluid: IL-6	Gr1 PBS	Gr2 PM _{2.5}	Gr3 TM5614	Gr4 PM _{2.5} + TM5614	3.1 & 3.2	
B. 	Thrombin, Heart and Lung tissues: Mac3, pSTAT3, Caspase3, VCAM-1	+/-	++	+/-	+		3.1, 3.3 & 3.4
C. 	Systolic and Diastolic Blood pressure, Cardiac Hypertrophy, Vascular Thrombosis	+/-	++	+/-	+		
Genetic deficiency of PAI-1	Endpoint Measurements	Results			Sections		
D. 	Lung tissue: Thrombosis	PAI-1 ^{+/+} PBS PM _{2.5}	PAI-1 ^{+/-} PBS PM _{2.5}	PAI-1 ^{-/-} PBS PM _{2.5}	3.7		
		- ++	- +/-	- ++			
PAI-1 Inhibition in Cellular Model	Endpoint Measurements	Results				Sections	
E. 	Collagen, PAI-1, SREBP1, SREBP2,	DMSO +/-	TM5614 +/-	PM _{2.5} ++	PM _{2.5} +TM5614 +/-	3.8	
E. 	Collagen, Fibronectin Nrf2	+/-	+/-	++	+/-		
		+	+	-	+		

overnight at room temperature, followed by fixation in 70% ethanol at 4 °C overnight, and then processed for embedding in paraffin. Paraffin-embedded heart and lung tissues were subjected to microtome sectioning and processed for histological analysis. For morphology studies, the tissues were processed for hematoxylin and eosin (H&E) staining. The H&E staining of specimens was performed on a fully automated platform (Leica Autostainer XL) using Harris hematoxylin (Fisherbrand) and eosin (Leica). The levels of collagen deposition and thrombotic blood vessels in lung and heart tissues were determined by H&E and Masson trichrome staining. Photographs were taken with an Olympus DP71 camera. The levels of matrix deposition were estimated by Fiji-ImageJ software.

2.6. Immunohistochemistry

For immunohistochemical studies, formalin-fixed paraffin-embedded tissue sections (4 µm) were first deparaffinized on an automated platform (Leica Autostainer XL). After deparaffinization, slides were subjected to an antigen retrieval step. Tissue sections were incubated with specific primary antibody overnight at 4 °C. The primary antibodies used in this study included cluster of differentiation 107 b (Mac3) (BD Biosciences), phospho-signal transducer and activator of transcription-3 (pSTAT3) (Cell Signaling), Cleaved Caspase 3 (Cell Signaling), vascular cell adhesion molecule 1 (VCAM1) (Abcam). Secondary antibody incubation and chromogenic reactions were performed using an automated system (Biocare Intellipath). After antibody staining, tissue sections were counterstained with hematoxylin. Slides were then coverslipped using a xylene-based mounting medium (Leica Micromount). Photographs were taken using a light microscope. The sum of several fields per lung and heart tissue from each mouse were used for staining intensity measurement using ImageJ software, and the

levels of statistical significance were determined.

2.7. Cell cultures and treatment with PM_{2.5} and TM5614

Primary cultures of human cardiac fibroblasts were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin. To test the efficacy of PAI-1 inhibitor TM5614 in suppression of PM_{2.5}-induced cardiac fibroblast dysfunction, confluent cultures of human cardiac fibroblasts were subcultured in 12-well clusters. After 24 h, media were replaced with 0.1% FBS-containing DMEM for 3 h. Cells were pretreated with TM5614 (10 µM) or DMSO (vehicle control) for 2 h followed by treatment with PM_{2.5} (50 µg/ml) in triplicate for another 2 h. For the other set of experiments, cells were pretreated with TM5614 (10 µM) or DMSO in triplicate for 24 h. After 24 h, media were replaced with 0.1% FBS-containing DMEM and treated with TM5614 (10 µM) or DMSO and PM_{2.5} (50 µg/ml) (dose selected based on published literature) (Liu et al., 2018; Zhu et al., 2018) in triplicate for another 24 h. In vitro experimental plan has been presented in Table 1 under E.

2.8. Preparation of cell lysates and Western blot analysis

At the end of treatment with PM_{2.5} in the absence and presence of PAI-1 inhibitor TM5614, supernatants from control and treated wells were collected. The cell lysates were prepared using RIPA lysis buffer (25 mM Tris•HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) (ThermoFisher Scientific) containing protease inhibitor cocktail (cOmplete) and phosphatase inhibitor cocktail (PhosSTOP) (Sigma). Cell lysates were pooled from 3 wells for each control and treatment group and equal amounts of protein were subjected to gel electrophoresis, transferred to PVDF membranes, and

subjected to western blotting using antibodies against PAI-1 (Molecular Innovations, Inc.), type I collagen (Southern Biotech), fibronectin (Millipore), sterol regulatory element binding protein-1 and 2 (SREBP1, SREBP2), nuclear factor erythroid related factor 2 (Nrf2) (Abcam), and α -tubulin (GenScript), with HRP-tagged corresponding secondary antibodies. The membranes were developed with enhanced chemiluminescence reagents (Luminata Forte, Millipore, Billerica, MA), and images of protein bands were captured using a BIO-RAD ChemiDoc XRS system (BIO-RAD, CA).

2.9. Statistical analysis

Data are presented as mean \pm SEM. The significance of differences between controls and experimental groups was determined by statistical analysis using Student's t-test, where a value of $P < 0.05$ was considered statistically significant. Statistical analyses were performed with GraphPad Prism (GraphPad Software Inc., San Diego, CA).

3. Results

3.1. PAI-1 inhibitor TM5614 reduces PM_{2.5} exposure-induced plasma levels of PAI-1 and thrombin in mice

Four groups of mice described in the Methods section were used in this study: group 1, controls; group 2, PM_{2.5} only; group 3, TM5614 only; and group 4, TM5614 and PM_{2.5}. After 24 h or 72 h of PM_{2.5} exposure, mice were sacrificed, and plasma was used for PAI-1 and TAT assays. The results revealed that exposure to PM_{2.5} (50 μ g/mouse) for 24 h increases the levels of PAI-1 significantly, and TAT complex insignificantly in plasma. Importantly, pretreatment of mice with PAI-1 inhibitor TM5614 completely abrogates the PM_{2.5}-induced increased levels of PAI-1 and TAT in plasma (Fig. 1A and B). Similarly, exposure to PM_{2.5} (200 μ g/mouse) for 72 h significantly stimulates the levels of TAT in plasma, and TM5614 abrogates that stimulation (Fig. 1C). These results indicate that PAI-1 inhibitor TM5614 is highly efficient in reduction of PM_{2.5}-induced PAI-1 and thrombin, which contribute to vascular pathologies like thrombosis.

3.2. PAI-1 inhibitor TM5614 significantly inhibits PM_{2.5}-induced IL-6 in BALF

BALF collected from the four groups of mice was used for determination of IL-6 levels as described in the Methods. Administration of

PM_{2.5} (50 μ g/mouse) for 24 h significantly increases the levels of pro-inflammatory cytokine IL-6 in BALF and most importantly, PM_{2.5} fails to stimulate the levels of IL-6 in mice fed with TM5614, indicating the efficacy of PAI-1 inhibitor TM5614 in amelioration of PM_{2.5}-induced lung inflammation (Fig. 1D). The levels of IL-6 in BALF from TM5614-treated (Group 3) and PBS control (Group 1) mice are not significantly increased ($p = 0.1301$).

3.3. PAI-1 inhibitor TM5614 alleviates PM_{2.5}-induced lung inflammation, apoptosis, and vascular pathologies

Wild-type mice were exposed to a single dose of PM_{2.5} (200 μ g/mouse). After 72 h, lung tissues were collected and processed for morphological analysis by H&E staining and immunohistochemical analysis using antibodies against inflammatory markers Mac3 and pSTAT3, apoptotic marker cleaved caspase 3, and adhesion molecule VCAM1. Morphologically, PM_{2.5}-exposed lungs (4 out of 7) are relatively denser compared to PBS controls (1 out of 4) and importantly, TM5614 partially reverses the PM_{2.5}-induced lung morphological changes (Supplemental Figure S1, representative images are presented). Immunohistochemistry results revealed the presence of low levels of inflammation in PBS-treated lungs as expected in fluid instilled-lungs. However, administration of PM_{2.5} increases the staining intensity of pSTAT3 and Mac3 compared to controls and mice treated with TM5614 alone. Treatment with PAI-1 inhibitor TM5614 modestly reduces PM_{2.5}-induced pSTAT3 without reaching statistical significance (representative images are presented in Fig. 2A and quantitative data are shown in Fig. 2B) and significantly reduces the Mac3 (representative images are presented in Fig. 2C, and quantitative data are shown in Fig. 2D) levels in lung tissues. These results are consistent with our observation that PM_{2.5} induces the level of pro-inflammatory cytokine IL-6 in BALF and TM5614 completely abrogates the PM_{2.5}-induced increased levels of IL-6 in lungs.

Exposure to PM_{2.5} for 72 h significantly increases the levels of cleaved caspase 3 positivity in lungs (Fig. 3A, representative image from each group; quantitative data are shown in Fig. 3B). Importantly, TM5614 treatment reduces the levels of cleaved caspase 3 positivity in lungs. Next, we determined the levels of adhesion molecule VCAM1, known to be involved in inflammation and vascular dysfunction, in PM_{2.5}-exposed murine lungs. Exposure to PM_{2.5} induces the levels of VCAM1, consistent with the previous observation that PM_{2.5}-induced stress increases VCAM1 in endothelial cells (Rui et al., 2016; Cui et al., 2019). Most importantly, TM5614 prevents PM_{2.5}-induced induction of

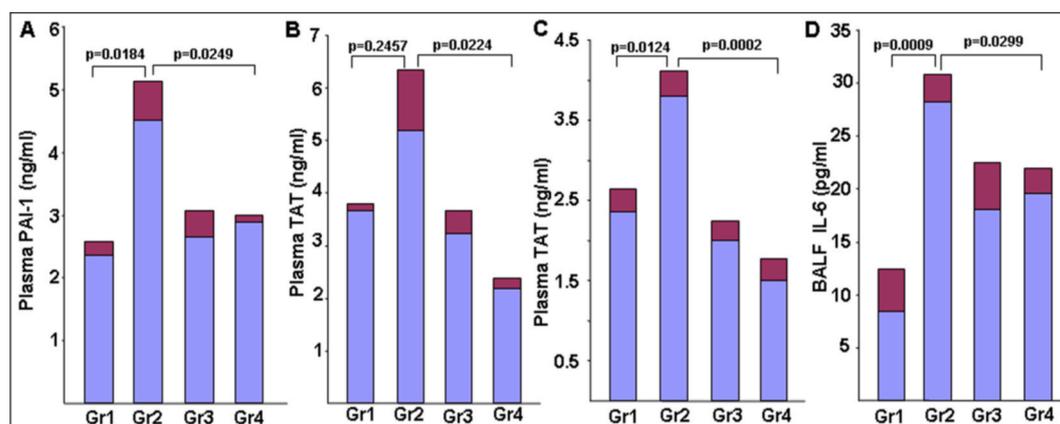


Fig. 1. PAI-1 inhibitor TM5614 inhibits the levels of PM_{2.5}-induced PAI-1 and TAT in plasma and IL-6 in BALF. Plasma collected from 4 groups of mice ($n = 4-8$) was used in duplicate for PAI-1 and TAT assays. Day 1-6: TM5614 (10 mg/kg/day); Day 7: PM_{2.5} (50 μ g/mouse) or PBS intratracheal instillation; Day 8: Plasma collected and processed for PAI-1 (A) or TAT (B) assay using an ELISA kit. (C) TAT levels in plasma from mice after 72 h treatment with PM_{2.5} (200 μ g/mouse) and TM5614 (10 mg/kg/day) ($n = 4-7$). (D) IL-6 levels in BALF from mice after 24 h treatment with PM_{2.5} (50 μ g/mouse) and TM5614 (10 mg/kg/day) were determined in duplicate using an ELISA kit ($n = 7-8$). Data presented as Mean (blue bar) \pm SEM (red bar). Gr1: PBS; Gr2: PM_{2.5}; Gr3: TM5614; Gr4: TM5614 + PM_{2.5}. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

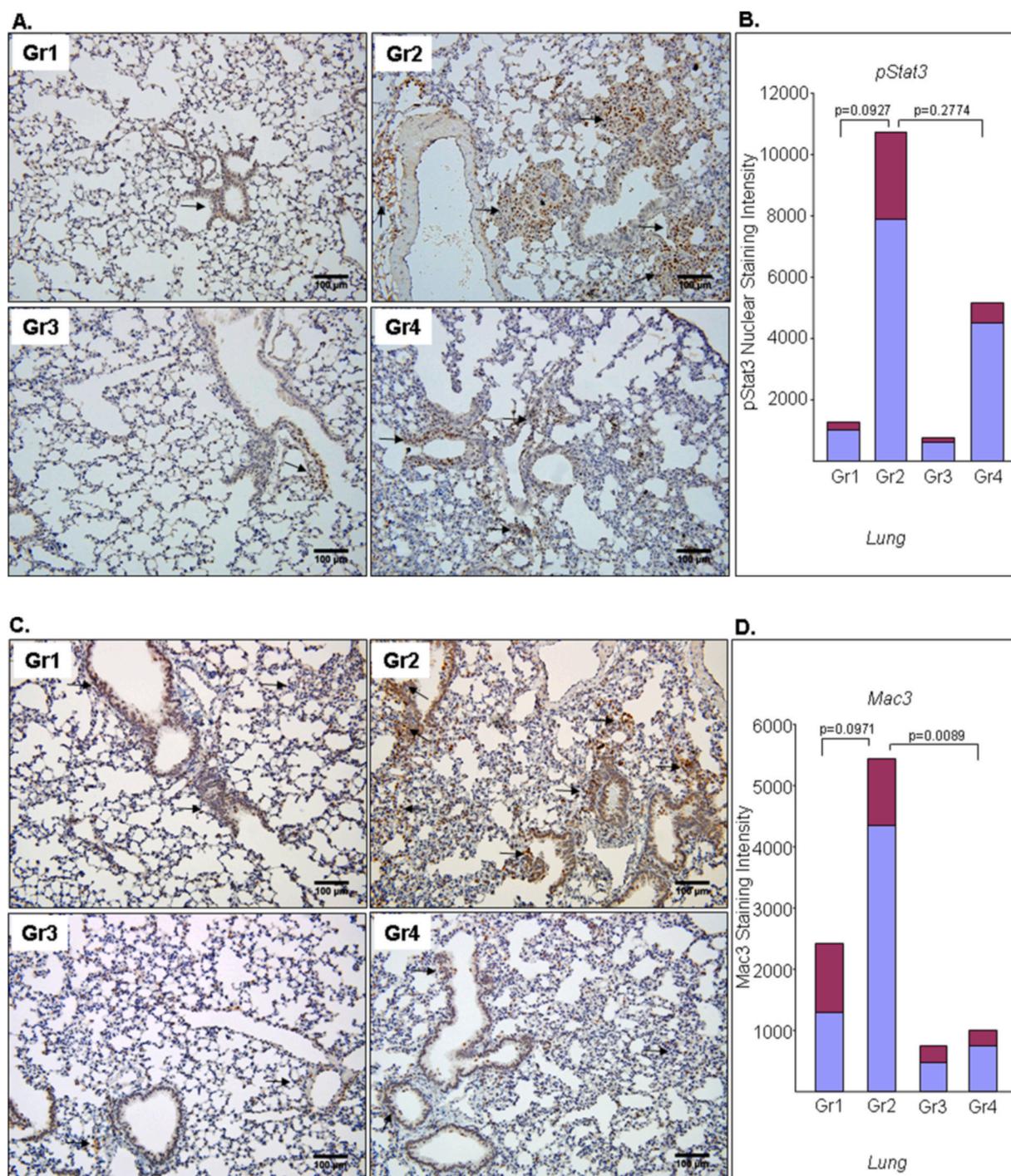


Fig. 2. Effect of PAI-1 inhibitor TM5614 on PM_{2.5}-induced inflammation in lungs. Lungs collected from 4 groups of mice (n = 4–6) were processed for immunohistochemistry using anti-pSTAT3 antibody (A,B) and antiMac3 antibody (C,D). Day 1–6: TM5614 (10 mg/kg/day); Day 7: PM_{2.5} (200 μg/mouse) or PBS instillation; Day 10: Lungs were collected and processed for immunohistochemistry. Representative images of pSTAT3 stained lung sections (A) and Mac3 stained lung sections (C) are shown. Images are reduced form of original 20X images. The levels of nuclear pSTAT3 in several fields of each lung section were determined by ImageJ software followed by statistical analysis. Quantitative data are shown in (B) for pSTAT3 and (D) for Mac3. Data presented as Mean (blue bar) ± SEM (red bar). Gr1: PBS; Gr2: PM_{2.5}; Gr3: TM5614; Gr4: TM5614 + PM_{2.5}. Arrows indicate the presence of pSTAT3 (A) and Mac3 (B) positive cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

VCAM1 (representative images are presented in Fig. 4A from each group; quantitative data are shown in Fig. 4B). Collectively, these results indicate that pharmacological inhibition of PAI-1 is an ideal approach to control air pollutant-induced inflammation and cellular apoptosis in murine lungs and that small molecule TM5614 effectively prevents air pollutant-induced lung pathological events.

3.4. Effect of PAI-1 inhibitor TM5614 on PM_{2.5}-induced cardiac inflammation and apoptosis

Hearts collected from the four groups of mice were processed for H&E staining and immunohistochemical analysis using antibodies against pSTAT3, Mac3, cleaved caspase 3, and VCAM1. There were no morphological changes in PM_{2.5}-exposed hearts (Supplemental Figure

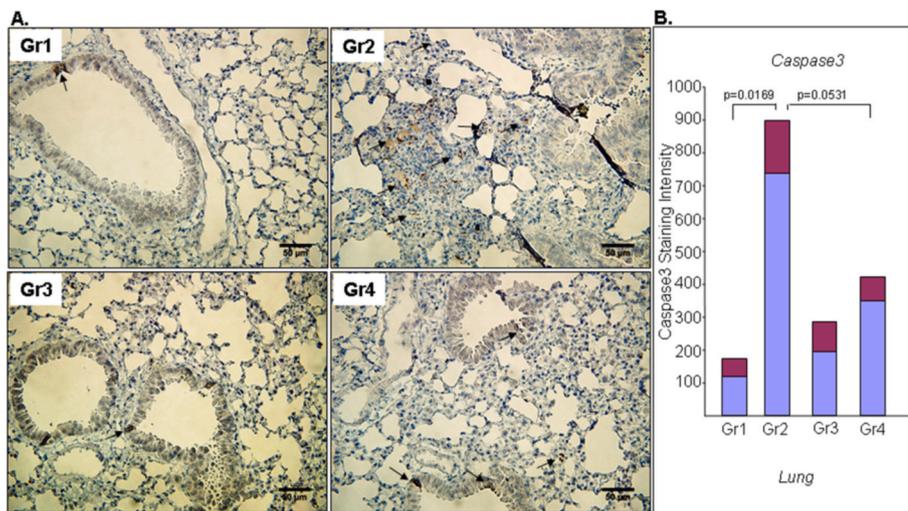


Fig. 3. Effect of TM5614 on PM_{2.5}-induced cellular apoptosis in lungs. Lungs collected from 4 groups of mice (n = 4–6) were processed for immunohistochemistry using an anti-cleaved caspase 3 antibody. Mice were fed with TM5614 (10 mg/kg/day) for 6 days and on day 7, PM_{2.5} (200 µg/mouse) instillation or PBS was used as control. After 72 h, lungs were processed for immunohistochemistry. Representative images are reduced form of original 40X images (A). The levels of cleaved caspase 3 in several fields of each lung were determined by ImageJ software. Quantitative data are shown in (B). Data are presented as Mean (blue bar) ± SEM (red bar). Gr1: PBS; Gr2: PM_{2.5}; Gr3: TM5614; Gr4: TM5614 + PM_{2.5}. Arrows indicate the presence of cleaved caspase 3 positive cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

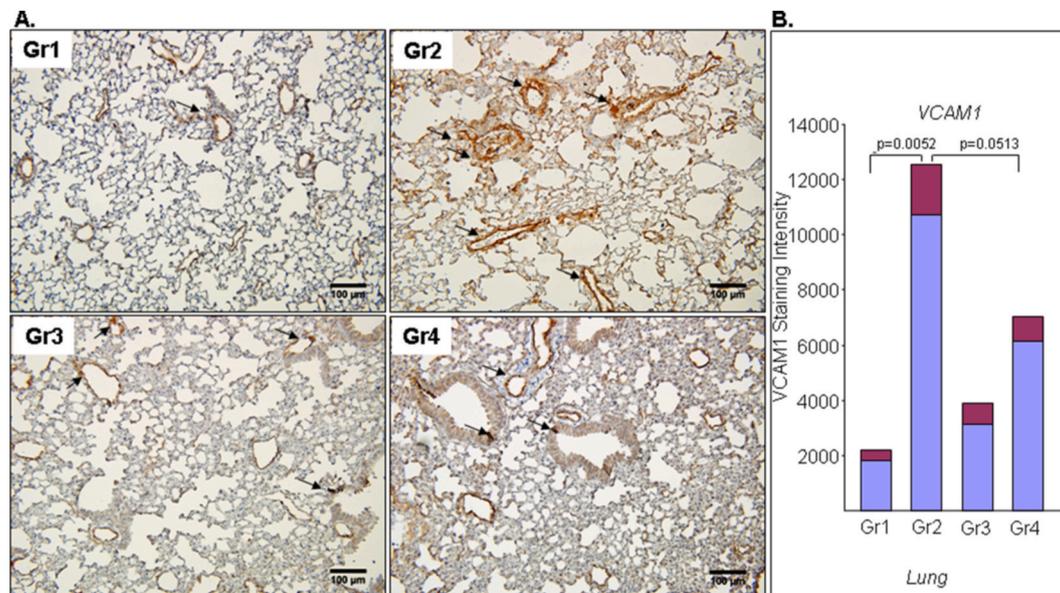


Fig. 4. TM5614 reduces PM_{2.5}-induced adhesion molecule VCAM1 in lungs. Lungs collected from 4 groups of mice (n = 4–6) were processed for immunohistochemistry using an anti-VCAM1 antibody. Day 1–6: TM5614 (10 mg/kg/day); Day 7: PM_{2.5} (200 µg/mouse) or PBS instillation; Day 10: Lungs were processed for immunohistochemistry. Representative images are reduced form of original 20X images (A). The levels of VCAM1 in several fields of each lung were determined by ImageJ. Quantitative data are shown (B). Data are presented as Mean (blue bar) ± SEM (red bar). Gr1: PBS; Gr2: PM_{2.5}; Gr3: TM5614; Gr4: TM5614 + PM_{2.5}. Arrows indicate the presence of VCAM1 positive sites. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

s2). Immunohistochemistry results revealed that exposure to PM_{2.5} (200 µg/mouse) for 72 h modestly increases the levels of cleaved caspase 3 in myocardial tissues and that TM5614 treatment reduces the levels of cleaved caspase 3 without attaining statistical significance (Fig. 5A, representative image from each group; quantitative data are shown in Fig. 5B). The levels of PM_{2.5}-induced apoptotic cells in hearts are lower compared to lungs. While administration of PM_{2.5} significantly increases cardiac inflammation as evidenced by increased Mac3 staining, TM5614 modestly reduces inflammation (Fig. 5C, representative image from each group; quantitative data are shown in Fig. 5D). There is no difference in the levels of pSTAT3 in cardiac tissues derived from control and treated mice (Supplemental Figure s3A, representative image of each group, and quantitative data are shown in Figure s3B). While exposure to PM_{2.5} modestly stimulates the levels of VCAM1 in myocardial tissues, in the TM5614-treated mice, PM_{2.5} fails to stimulate adhesion molecule VCAM1 (Fig. 6A, representative image from each

group; quantitative data are shown in Fig. 6B). These results signify the efficacy of PAI-1 inhibitor TM5614 in suppression of air pollutant-induced cardiovascular pathologies.

3.5. Effect of long-term exposure to PM_{2.5} on blood pressure, cardiac hypertrophy, and cardiac matrix remodeling

Mice were divided into 4 groups. In Group1, mice were fed with regular chow and received PBS. Group 2, mice were fed with regular chow and received PM_{2.5} (100 µg/mouse/once per week for 4 weeks). Group 3, mice were fed with chow containing TM5614 (10 mg/kg) for 6 days and then received PBS for 4 weeks. Group 4, mice were fed with chow containing TM5614 (10 mg/kg) for 6 days and then received PM_{2.5} (100 µg/mouse/once per week for 4 weeks). At the end of the 4 weeks, blood pressure was determined, and each mouse was subjected to echocardiography. The results revealed that 4 weeks of PM_{2.5} exposure

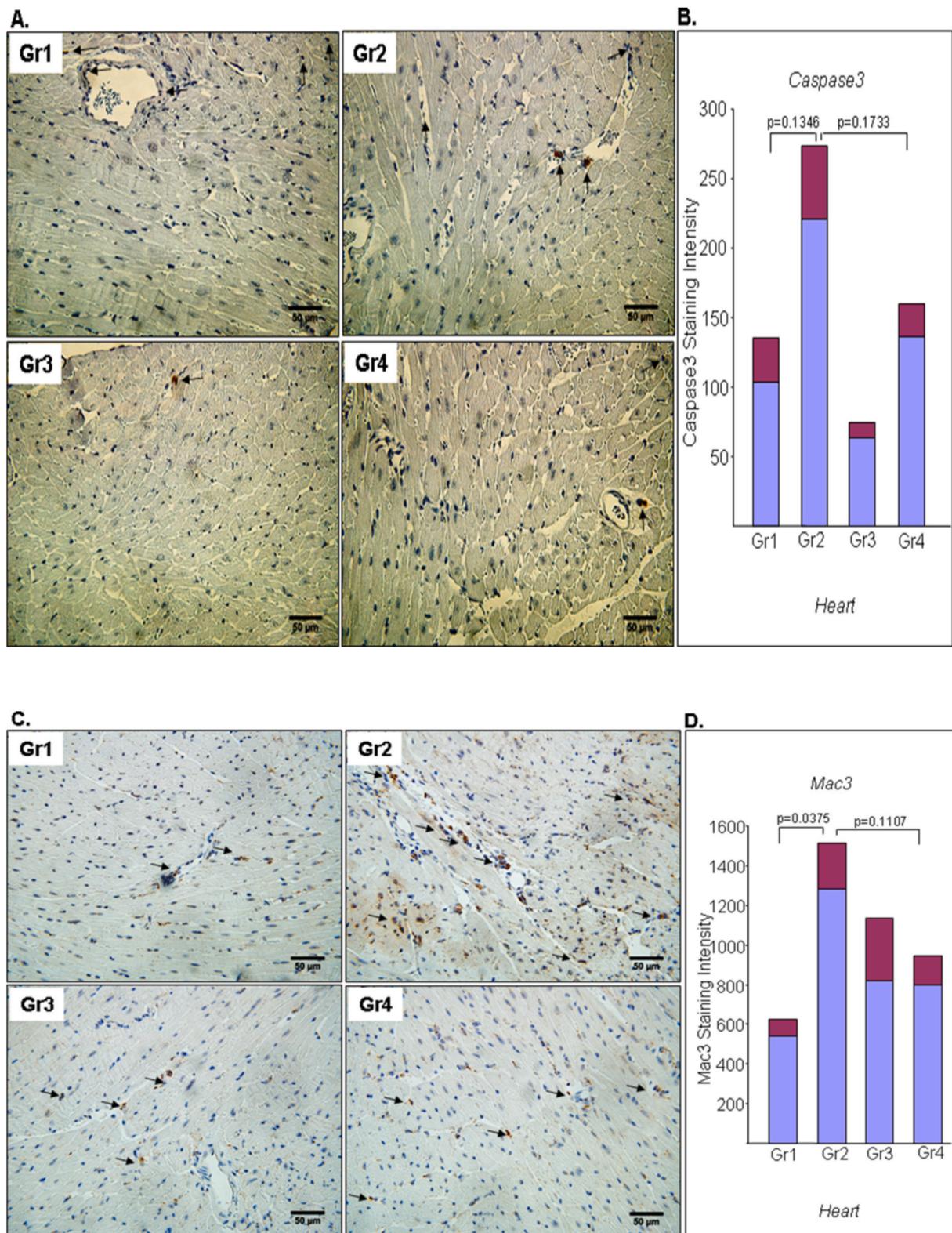


Fig. 5. Effect of PAI-1 inhibitor TM5614 on PM_{2.5}-induced cellular apoptosis and inflammation in hearts. Hearts collected from 4 groups of mice (n = 4–6) were processed for immunohistochemistry using an anti-cleaved caspase 3 antibody (A) and anti-Mac3 antibody (C). Day 1–6: TM5614 (10 mg/kg/day); Day 7: PM_{2.5} (200 µg/mouse) or PBS instillation. After 72 h, hearts were collected and processed for immunohistochemistry. Representative images are reduced form of original 40X images. The levels of cleaved caspase 3 and Mac3 in several fields of each heart section were determined by ImageJ. Quantitative data are shown in (B) for cleaved caspase 3 and (D) for Mac3. Data are presented as Mean (blue bar) ± SEM (red bar). Gr1: PBS; Gr2: PM_{2.5}; Gr3: TM5614; Gr4: TM5614 + PM_{2.5}. Arrows indicate the presence of cleaved caspase 3 (A) and Mac3 (C) positive cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

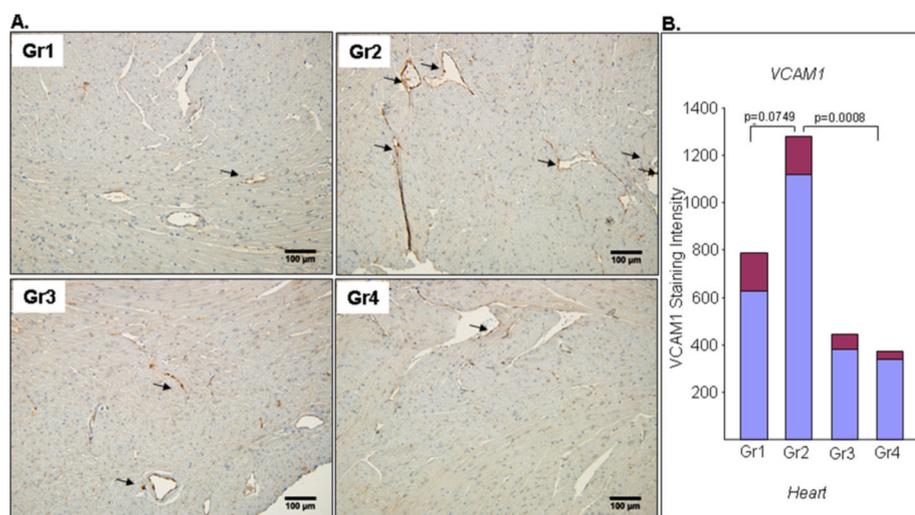


Fig. 6. TM5614 reduces PM_{2.5}-induced adhesion molecule VCAM1 in hearts. Hearts collected from 4 groups of mice (n = 4–6) as indicated were processed for immunohistochemistry using an anti-VCAM1 antibody. Day 1–6: TM5614 (10 mg/kg/day); Day 7: PM_{2.5} (200 µg/mouse) or PBS instillation; Day 10: Heart sections were processed for immunohistochemistry. Representative images are shown in (A). Images are reduced form of original 20X images. The levels of VCAM1 in several fields of each heart were determined by ImageJ software. Quantitative data are shown in (B). Data are presented as Mean (blue bar) ± SEM (red bar). Gr1: PBS; Gr2: PM_{2.5}; Gr3: TM5614; Gr4: TM5614 + PM_{2.5}. Arrows indicate the presence of VCAM1 positivity. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

increases systolic pressure non-significantly and diastolic blood pressure significantly. Importantly, treatment of mice with PAI-1 inhibitor TM5614 reduces the PM_{2.5}-induced blood pressure increase (Supplemental Figure s4A,B). Analysis of echocardiography data revealed that there is no significant differences in EF, FS, left ventricular anterior or posterior wall thickness-systolic or diastolic (LVAWs, LVAWd, LVPWs and LVPWd) within control and treated groups (data not shown). Post-mortem heart weight analysis reveals that PM_{2.5} (100 µg/mouse once per week for 4 weeks) induces cardiac hypertrophy based on increased heart weight/body weight ratio and that TM5614 decreases the PM_{2.5}-induced cardiac hypertrophy (Supplemental Figure s4C). Collectively, these results suggest that TM5614 is effective in amelioration of PM_{2.5}-induced cardiac pathologies including hypertension and cardiac hypertrophy. However, PM_{2.5} fails to stimulate fibrogenesis in heart tissue after 4 weeks of treatment (100 µg once per week for 4 weeks) (data not shown).

3.6. PAI-1 inhibitor TM5614 reduces long-term PM_{2.5} exposure-induced pulmonary and cardiac thrombosis in mice

Mice were divided into 4 groups and treated for 4 weeks as described above. At the end of treatment, mice were sacrificed, and heart and lungs were collected. Lung and heart tissues were processed for H&E staining and Masson's trichrome staining. Exposure to PM_{2.5} for 4 weeks significantly induces pulmonary thrombogenesis in mice (incidence of thrombus formation: 4 out of 7) compared to PBS controls (incidence of thrombus formation: 0 out of 4) and mice treated with TM5614 alone (incidence of thrombus formation: 0 out of 5). Most importantly, PM_{2.5} failed to induce pulmonary thrombosis in mice pretreated with PAI-1 inhibitor TM5614 (incidence of thrombus formation: 0/4) (Fig. 7, representative image of each group). This result is highly significant, as it clearly indicates the beneficial effect of PAI-1 inhibitor TM5614 in improving vascular thrombosis, a major pulmonary pathology, in mice exposed to air pollutant PM_{2.5}. However, PM_{2.5} fails to stimulate the pulmonary fibrogenesis significantly after 4 weeks of treatment

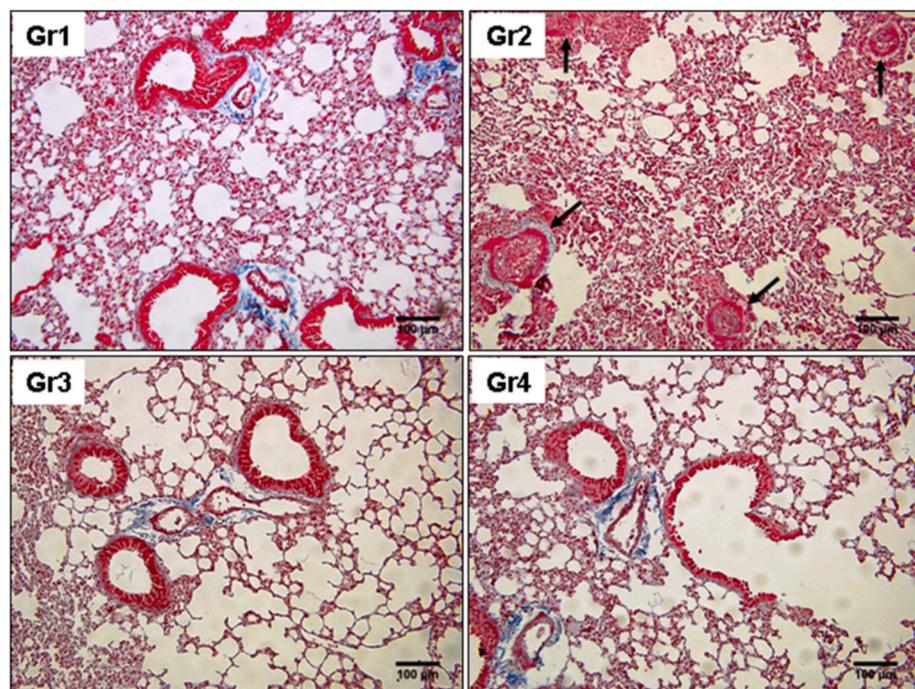


Fig. 7. PM_{2.5} induces pulmonary thrombosis and PAI-1 inhibitor TM5614 prevents lung thrombogenesis. Mice were divided into 4 groups (n = 4–7). In Group1, mice were fed with regular chow and received PBS. In Group 2, mice were fed with regular chow and received PM_{2.5} (100 µg/mouse/once per week for 4 weeks). In Group 3, mice were fed with chow containing TM5614 (10 mg/kg/day) for 6 days and then received PBS. In Group 4, mice were fed with chow containing TM5614 (10 mg/kg/day) for 6 days and then received PM_{2.5} (100 µg/mouse/once per week for 4 weeks). Representative image from each group are shown. Images are reduced form of original 20X images. An arrow indicates the presence of a thrombus.

(Supplemental Figure s5A,B).

Analysis of hearts from each group revealed that longer exposure to PM_{2.5} modestly induces cardiac thrombosis in mice, to a lesser extent than in lungs. However, PM_{2.5} failed to induce cardiac thrombosis in mice pretreated with PAI-1 inhibitor TM5614 (Supplemental Figure s6). These results are consistent with our observation that PM_{2.5} induces the expression of PAI-1 and thrombin, the major contributors to thrombogenesis, and that TM5614 completely abrogates the PM_{2.5}-induced increased levels of PAI-1 and thrombin in plasma (Fig. 1). Additionally, these results further indicate that PAI-1 is an ideal druggable target for the treatment of air pollutant-induced cardiopulmonary pathologies, including vascular thrombosis.

3.7. PAI-1 heterozygous mice are partly protected from PM_{2.5}-induced pulmonary thrombosis

As a complementary study to pharmacological inhibition of PAI-1 and its beneficial effect on air pollutant-induced cardiopulmonary and vascular pathologies, next we investigated the effects of haplo- and complete genetic deficiency of PAI-1 on air pollutant PM_{2.5}-induced cardiopulmonary pathologies. Wild-type, PAI-1 heterozygous, and PAI-1 knockout mice were treated with PM_{2.5} (50 µg/mouse/week for 4 weeks) or PBS as a control. All mice were fed with regular chow. Four weeks of PM_{2.5} exposure induces pulmonary thrombosis in both wild-type and PAI-1 knockout mice, indicating complete deficiency of PAI-1 does not protect against developing PM_{2.5}-induced pulmonary pathologies. Interestingly, PAI-1 heterozygous mice are partially protected from PM_{2.5}-induced pulmonary thrombotic pathogenesis, indicating that, like pharmacological inhibition of PAI-1, PAI-1 haplodeficiency, but not complete absence of PAI-1, is beneficial for air pollutant-induced pulmonary pathologies (Supplemental Figure s7). However, we did not observe any difference in pulmonary matrix remodeling in PM_{2.5}-treated wild-type, PAI-1 heterozygous, and knockout groups as evidenced by Masson's trichrome staining (data not shown).

3.8. TM5614 reduces PM_{2.5}-induced cellular stresses and abnormalities

Previous reports suggest that PM_{2.5} can enter into the systemic circulation and affects cardiovascular system (Nelin et al., 2012). As PM_{2.5} is a known inducer of reactive oxygen species (ROS), TGF-β, and matrix remodeling, and fibroblasts are the major contributing cell type in matrix protein production, we have developed an in vitro model system to study the effects of PM_{2.5} on cellular processes in cardiac fibroblasts and the efficacy of TM5614 in ameliorating PM_{2.5}-induced cellular abnormalities. Our results showed that PM_{2.5} modestly induces the levels of PAI-1, type I collagen, and fibronectin and that TM5614 reduces the PM_{2.5}-induced stimulation of these fibrogenic factors (Fig. 8A–C). The PM_{2.5}-induced stimulation of PAI-1 and its normalization by TM5614 in vitro is consistent with the in vivo observation on plasma levels of PAI-1 in PM_{2.5}- and TM5614-treated mice (Fig. 1). As SREBP1, a basic-helix-loop-helix leucine zipper family transcription factor, mediates profibrogenic signaling and fibrogenesis (Chen et al., 2014), and air pollutants induce the expression of SREBP1 (Yan et al., 2020), we measured the levels of SREBP1 and SREBP2 in control, PM_{2.5}-, and TM5614-treated cardiac fibroblasts. Interestingly, PM_{2.5} induces the expression of SREBP1/2 in human cardiac fibroblasts and most importantly, TM5614 completely abrogates that stimulation, indicating that TM5614 effectively blocks PM_{2.5}-induced profibrogenic signaling through suppression of SREBP1 and SREBP2 (Fig. 8A). As PM_{2.5} induces reactive oxygen species (Liu et al., 2018) and Nrf2, a basic leucine zipper (bZip) transcription factor, is a master regulator of antioxidant genes and signaling (Vomund et al., 2017), we measured the effect of PM_{2.5} on Nrf2 expression in human cardiac fibroblasts and the efficacy of TM5614 in normalizing the Nrf2 expression. Our results revealed that PM_{2.5} reduces the levels of Nrf2 in cardiac fibroblasts and importantly, that TM5614 reverses the suppression of Nrf2 by PM_{2.5} (Fig. 8C).

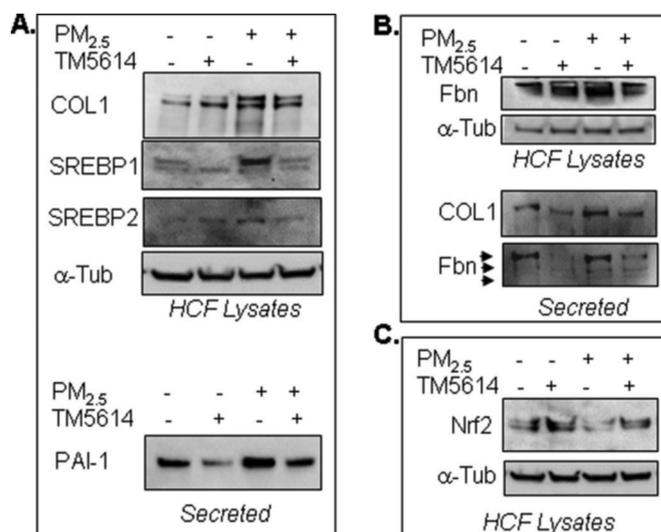


Fig. 8. TM5614 blocks PM_{2.5}-induced cellular stress and profibrogenic responses. Human cardiac fibroblasts (HCF) were cultured in 12-well clusters. After 24 h, media were replaced with 0.1% FBS containing DMEM for 3 h. Cells were pretreated with TM5614 (10 µM) or DMSO (vehicle control) for 2 h followed by treatment with PM_{2.5} (50 µg/ml) in triplicate for another 2 h. Supernatants and cell lysates were used for western blotting using antibodies as indicated (A). In (B) and (C), cells were pretreated with TM5614 (10 µM) or DMSO in triplicate. After 24 h, media were replaced with 0.1% FBS containing DMEM and treated with TM5614 (10 µM) or DMSO and PM_{2.5} (50 µg/ml) in triplicate for another 24 h. Supernatants and cell lysates were processed for western blotting using antibodies as indicated (B, C). Fbn: fibronectin; COL1: Type 1 collagen; α-Tub: α-Tubulin.

Collectively, these in vitro results indicate the efficacy of PAI-1 inhibitor TM5614 in improvement of cardiac cellular stresses and abnormalities induced by exposure to air pollutant PM_{2.5}.

4. Discussion

Environmental pollution is a global threat to organismal growth and health. Short-term or long-term exposure to environmental pollutants like PM causes devastating CPVDs. CPVDs including pulmonary inflammation, vascular dysfunction, hypertension, arteriosclerosis, arrhythmias, and myocardial infarction cause an estimated 60–80% of environmental pollutant-associated deaths worldwide (Brook et al., 2004, 2010; Nel, 2005; Simkhovich et al., 2009; Franchini and Manucci, 2011; Budinger and Mutlu, 2011; Du et al., 2016; Bourdrel et al., 2017). However, the underlying molecular basis of PM-induced CPVDs is not well understood, and an effective therapeutic approach to block PM-induced CPVD-related pathologies is not available. As exposure to environmental pollutants deregulates the plasminogen activator system (Upadhyay et al., 2010; Budinger et al., 2011), and PAI-1 plays pivotal roles in initiation and progression of CPVDs (Westrick and Eitzman, 2007; Ghosh and Vaughan, 2012; Ghosh et al., 2016; Vaughan et al., 2017; Sun et al., 2019), we initiated the present study in order to delineate the role of PAI-1 in air pollutant PM_{2.5}-induced CPVD-related pathologies, and assess the potential of PAI-1 as a druggable target for CPVD therapy. Using the small molecule inhibitor TM5614, which targets PAI-1, and mouse strains with PAI-1 haplo- or complete deficiency, the present study demonstrated that PAI-1 plays a key role in air pollutant PM_{2.5}-induced CPVD pathologies and that pharmacological inhibition of PAI-1 ameliorates PM_{2.5}-induced CPVD pathologies.

In this study, we demonstrated that short-term exposure to air pollutant PM_{2.5} increases the levels of pro-inflammatory and pro-thrombotic PAI-1 and thrombin in plasma and inflammatory cytokine IL-6 in lungs, consistent with previous observations (Budinger et al., 2011). The levels of TAT complex serve as an indicator of coagulation

activation. Thrombin is a serine protease that converts soluble fibrinogen to an insoluble fibrous protein, fibrin that polymerizes and becomes a key component of blood clots. Antithrombin, an endogenous inhibitor of thrombin, forms the TAT complex with thrombin. As thrombin is highly unstable and has a short half-life in plasma, the TAT complex is measured as an indicator of thrombin levels and activation of coagulation that contributes to thrombosis (Chen et al., 2019).

Most importantly, here we have presented data showing that the stimulation of plasma levels of PAI-1 and thrombin, and BALF levels of IL-6 in air pollutant-exposed mice is abrogated by treatment with PAI-1 inhibitor TM5614. The elevated levels of PAI-1 and IL-6 clearly indicate increased inflammation by exposure to air pollutant PM_{2.5}, and early inflammation plays a pivotal role in the development of downstream inflammatory signals and organ pathologies, especially in the lungs and cardiovascular system, including vascular occlusion and disruption of lung and heart tissue homeostasis (Fig. 9). Suppression of IL-6 by PAI-1 inhibitor TM5614 can be explained in the light of previous observations made by Kubala and colleagues, who demonstrated that while elevated PAI-1 stimulates the levels of IL-6 and activates downstream pSTAT3, depletion of PAI-1 is associated with down-regulation of IL-6 and pSTAT3 activation in response to inflammatory signals (Kubala et al., 2018).

Next, we investigated the effect of short-term exposure to PM_{2.5} on lung and heart inflammation and apoptosis and showed that the lung morphology is abnormal in several PM_{2.5}-exposed mice (200 µg/mouse) in terms of its tissue density, consistent with previous observations that PM_{2.5} causes damage in lung morphological structure (Urich et al., 2009; Budinger et al., 2011; Yang et al., 2019). These results indicate that acute exposure to air pollutant is able to damage lung tissue architecture. However, in the presence of PAI-1 inhibitor TM5614, PM_{2.5} fails to induce pulmonary tissue damage. Furthermore, TM5614 reduces air pollutant PM_{2.5}-induced inflammation, apoptosis, and vascular dysfunction as evidenced by the decreased levels of inflammation- and apoptosis-associated factors in lungs derived from mice exposed to PM_{2.5} in the presence of TM5614. Similarly, TM5614 is also effective in suppression of PM_{2.5}-induced modestly elevated inflammatory and apoptotic events in murine hearts. These results signify that pharmacological neutralization of PM_{2.5}-induced PAI-1 is an effective approach for suppression of early events of air pollutant-induced cardiovascular and pulmonary pathological events. Previous studies demonstrated that PM_{2.5} induces the levels of PAI-1 (Upadhyay et al., 2010; Budinger et al., 2011), and that elevated PAI-1 plays a key role in a number of sub-clinical and clinical pathologies, including inflammation, multi-organ fibrosis, atherosclerosis, insulin resistance, obesity, and multi-morbidities (Westrick and Eitzman, 2007; Cesari et al., 2010; Ghosh and Vaughan, 2012; Vaughan et al., 2017). The elevated PAI-1 may also contribute to cellular apoptosis in a context-dependent manner. For example, the elevated PAI-1 in alveolar type II cells derived from idiopathic pulmonary fibrosis patients is associated with elevated levels of cleaved caspase 3 and thus increased apoptosis (Marudamuthu et al., 2015). Therefore, pharmacological suppression or neutralization of PAI-1 is an ideal approach to control pathological/sustained inflammation and disease development like air pollutant-induced increased inflammation and apoptosis-driven cardiopulmonary diseases.

The most notable observation we made in the present study is the induction of significant levels of pulmonary and, to a lesser degree, cardiac vascular thrombosis in mice exposed to air pollutant PM_{2.5}, and that air pollutant exposure fails to develop pulmonary and cardiac thrombosis in the mice treated with PAI-1 inhibitor TM5614. This result is consistent with our observation that TM5614 significantly reduces PM_{2.5}-induced increased levels of inflammation and prothrombotic mediators IL-6, PAI-1, and thrombin. It is important to mention that inflammation-associated increased levels of inflammatory mediators like circulatory IL-6, PAI-1, and VCAM-1 in the vascular wall are known key contributors to endothelial dysfunction, and vascular remodeling that leads to the initiation and progression of cardiovascular pathologies

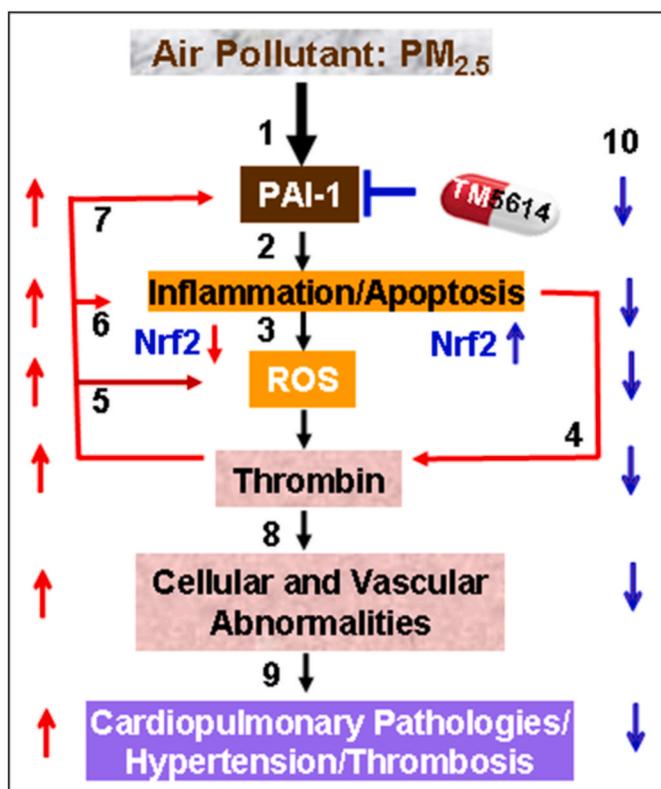


Fig. 9. Possible pathological pathways driven by PM_{2.5}-induced PAI-1 and the beneficial effect of PAI-1 inhibitor TM5614. Air pollution stressors increase the levels of proinflammatory and prothrombotic mediators/regulators that cause cellular and vascular dysfunction and contribute to cardiopulmonary vascular pathologies. Neutralization of PM_{2.5}-induced PAI-1 with TM5614 reduces PM_{2.5}-induced cardiopulmonary vascular pathologies. In the depicted model, the individual step and feed-back loop are supported by the present study and or previous published works by other investigators. 1. Air-pollutant: PM_{2.5} increases the level of PAI-1 (present study and Upadhyay et al., 2010; Budinger et al., 2011); 2. PAI-1 increases inflammation and apoptosis (This study and Kubala et al., 2018); 3. Inflammation increases ROS (Mittal et al., 2014); 4. Inflammation induces thrombin (Margetic S. 2012, Foley and Conway, 2016); 5. Thrombin induces the levels of ROS (Carrim et al., 2015); 6. Thrombin increases inflammation/apoptosis (Lopez et al., 2007; Chen and Dorling, 2009; Danckwardt et al., 2013; Foley and Conway, 2016); 7. Thrombin increases PAI-1 level (Hsieh et al., 2019); 8. Thrombin induces cellular and vascular abnormalities (present study and Rabiet et al., 1994); 9. Increased cellular dysfunction, elevation of thrombin-induced fibrinogen to fibrin deposition leads to cardiopulmonary pathologies, hypertension, thrombosis (present study and Savoia et al., 2011), 10. The results of the present biochemical, histological, immunohistological and cellular studies provide evidence on the pivotal role of air-pollutant PM_{2.5}-induced PAI-1 in cardiopulmonary vascular pathologies, and the efficacy of a novel PAI-1 inhibitor TM5614 in improving air pollutant-induced cardiopulmonary vascular pathologies. Red upward arrow indicates induction of pathological factors by air-pollutants. Red downward arrow indicate downregulation of anti-pathological factor. Blue downward arrow indicates amelioration of pathological events by PAI-1 inhibitor TM5614. Blue upward arrow indicates upregulation of anti-pathological factor. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

including hypertension and cardiac hypertrophy (reviewed in Savio et al., 2011). Furthermore, normalization of PM_{2.5}-induced increased blood pressure and heart weight by TM5614 treatment are also significant in the context of the pivotal role of PAI-1 in PM_{2.5}-induced vascular pathologies. In this context, it is important to note that previous studies implicated PM and higher levels of PAI-1 in high blood pressure (Brook and Rajagopalan, 2009; Peng et al., 2017). Like wild-type mice, several PAI-1 knockout mice also developed pulmonary thrombosis upon longer

exposure to air pollutant PM_{2.5}. Interestingly, PAI-1 haplodeficient mice exposed to PM_{2.5} are partly protected from developing pulmonary thrombosis, indicating that, like pharmacological inhibition of PAI-1, partial genetic deficiency of PAI-1 or low levels of PAI-1 are beneficial for organisms exposed to air pollutants. Therefore, it is apparent that normalization of the levels of PAI-1, but not complete depletion, is favorable to prevent air pollutant-induced pulmonary pathologies. Further studies are necessary to understand the underlying molecular basis. Although previous reports suggested significant cardiac matrix remodeling in mice and rats in response to exposure to air pollution (Xu et al., 2019; Yue et al., 2019; Sun et al., 2020), under our experimental setup (100 µg/once/week/4 weeks), we did not observe any significant change in collagen deposition in PM_{2.5}-exposed murine lungs and hearts. However, our in vitro study showed up-regulation of profibrogenic signal transducers and matrix proteins in cardiac fibroblasts exposed to PM_{2.5}, and importantly, TM5614 is effective in the suppression of these PM_{2.5}-induced profibrogenic responses. Further studies are required to investigate the effect of PM_{2.5} exposure with an increased number of doses/week for 3–6 months on pulmonary and cardiac pathological matrix remodeling and whether TM5614 can effectively block PM_{2.5}-induced pulmonary and cardiac fibrogenesis on that time-scale. Together, these results signify the pivotal role of PAI-1 in air pollutant-induced cardiopulmonary vascular pathologies and establish the potential therapeutic efficacy of PAI-1 inhibitor TM5614, an FDA-approved drug for clinical trials of other PAI-1 related pathologies, in amelioration of air pollutant exposure-mediated cardiopulmonary pathologies.

In search of cellular and molecular events induced by exposure to air pollutant PM_{2.5}, we studied the effects of PM_{2.5} on cultured human cardiac fibroblasts in the presence and absence of TM5614. The rationale behind using cardiac fibroblasts in this cellular model study comes from previous experimental demonstrations that PM_{2.5} can enter into the systemic circulations and thus directly affects heart and blood vascular system (reviewed in Nelin et al., 2012). The in vitro results suggest that air pollutant PM_{2.5} modestly induces profibrogenic markers and regulators, namely profibrogenic PAI-1, matrix protein collagen, and transcriptional regulators SREBP1 and SREBP2; and most importantly, that PAI-1 inhibitor efficiently blocks air pollutant-induced increases in PAI-1, profibrogenic regulators, and target matrix proteins. The rescue of PM_{2.5}-induced suppression of Nrf2 levels by TM5614 is interesting, as it clearly indicates that suppression of Nrf2, a major transcriptional regulator of antioxidant genes, and augmentation of oxidative stress by PM_{2.5} can be prevented by pharmacological inhibition of PAI-1. This result is consistent with our previous observations showing PAI-1 inhibition is associated with the rescue of homocysteine-mediated suppression of the antioxidant regulator Nrf2 (Sun et al., 2019), and of doxorubicin-mediated suppression of antioxidant catalase (Ghosh et al., 2016), as well as with prevention of accelerated cellular aging.

5. Conclusion

The present preclinical study establishes that PAI-1 is a potential druggable target for the treatment of air pollutant PM_{2.5}-induced cardiopulmonary vascular pathologies including inflammation, thrombosis, hypertension, and cardiac hypertrophy. Most importantly, here we demonstrate that TM5614, a drug-like small molecule inhibitor of PAI-1, effectively ameliorates these cardiopulmonary vascular pathologies by suppression of air pollutant PM_{2.5}-induced mediators of inflammation, prothrombotic factors, profibrogenic regulators, and by rescuing PM_{2.5}-induced suppression of a key antioxidant regulator (Model depicting the PM_{2.5}-induced signaling pathway and feed-back loop in Fig. 9 which is based on results from the present study and previously published articles). Further in vitro studies are needed to test the efficacy of TM5614 in amelioration of air pollutant-induced other signaling pathways involved in cardiopulmonary vascular pathologies,

including inflammatory signaling, canonical or non-canonical TGF-β signaling, and pro-apoptotic signaling pathways in endothelial cells and macrophages. Future expansion of the present study will be helpful to design clinical trials of TM5614 for the treatment of air pollutant-induced CPVDs.

Author's contributions

Asish K Ghosh: Conceptualization, Methodology, Investigation, Visualization, Analysis, Writing of Original Draft, Reviewing, Editing, Funding Acquisition; Saul Soberanes, Elizabeth Lux, Meng Shang, Raul Piseaux Aillon, Mesut Eren: Methodology, Investigation, Data collection, Reviewing, Data Analysis; G.R. Scott Budinger, Toshio Miyata, Douglas E Vaughan: Provided Valuable Resources, Conceptualization, Reviewing, Interpretation, Funding Acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2021.117283>.

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