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Palmitoleic acid inhibits *Pseudomonas aeruginosa* quorum sensing activation and protects lungs from infectious injury

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Abstract

Background Unsaturated fatty acids targeting quorum sensing (QS) system have shown potential application in reducing bacterial virulence. We aim to investigate the effect of palmitoleic acid (PMA) on *P. aeruginosa* QS activation, and its impact on infection-induced lung injury.

Methods The influence of PMA on QS signaling molecule (3OC12-HSL and C4-HSL) concentrations, pyocyanin production, and QS gene transcription levels were examined in wildtype PAO1 culture. The roles of PMA in reducing infection-induced injury were assessed in human bronchial epithelial BEAS-2B cells and mouse lung infection models, respectively. PMA levels and QS signaling molecule concentrations were tested in the bronchoalveolar lavage fluid (BALF) of bronchiectasis patients with first-time detection of *P. aeruginosa* infection.

Results PMA administration dose-dependently suppressed the expression of QS signaling molecules, pyocyanin, and QS genes during the logarithmic stage of bacterial growth. In BEAS-2B cells, PMA-treated PAO1 filtrates significantly reduced cell apoptosis and expression of IL-8 and IL-6. In mouse lung infection models, prophylactically oral administration of PMA significantly downregulated the expression of *P. aeruginosa* QS signals and QS genes (*lasR*, *rhlR*, *rhlI*, *lasB*, *rhlA*, *phzA1*, *phnA*) in lungs, and relieved neutrophilic airway inflammation. Finally, PMA levels were negatively correlated with the concentrations of both 3OC12-HSL and C4-HSL in BALF of bronchiectasis patients, and positively correlated with their forced vital capacity (FVC) and forced expiratory volume in the first second ($FEV_{1.0}$).

Conclusion Our findings show that PMA inhibits *P. aeruginosa* QS activation and protects lungs from injury caused by bacterial virulence. Hence, PMA may serve as a potential anti-QS agent against *P. aeruginosa* infection and would help to alleviate lung injury in bronchiectasis patients.

Keywords Palmitoleic acid, *Pseudomonas aeruginosa*, Quorum sensing, Lung infection, Infection-induced injury

Introduction

Pseudomonas aeruginosa is a common gram-negative pathogen responsible for opportunistic infections in patients with chronic lung diseases such as bronchiectasis. *P. aeruginosa* can cause serious lung injuries by releasing numerous virulent factors regulated by quorum sensing (QS) systems [1, 2]. In recent years, eradicating *P. aeruginosa* has been increasingly challenging due to its antibiotic resistance. Hence, targeting QS system

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emerges as an important anti-infective strategy, as it can not only exert an anti-virulence effect but also avoid evolutionary pressure on bacterium that develop resistance [3]. *P. aeruginosa* QS signaling pathways are activated after signaling molecules outside the bacterial cells reach a threshold. These signaling molecules can diffuse through bacterial membranes and combine with their responding receptors in the cells. Among these molecules, *N*-3-oxo-dodecanoyl-homoserine lactone (3OC12-HSL) and *N*-butanoyl-homoserine lactone (C4-HSL) are critical signals that activate *P. aeruginosa* LasR-I and RhlR-I systems respectively, and promote the transcription of virulence genes [4].

Unsaturated fatty acids (uFA) are medium- or long-chain carboxylic acids containing one or more carbon–carbon double bonds, and exhibit significant antibacterial activities against various pathogens, including multidrug-resistant bacteria [5, 6]. In these years, uFA that targets QS system has shown potential application in reducing bacterial virulence and relieving infection-induced injury [7, 8]. Palmitoleic acid (PMA) is a cis-monounsaturated n-7 fatty acid consisting of 16 carbon atoms, classified within the omega-7 fatty acids group. So far, whether PMA could target QS system and exert an anti-infection effect on *P. aeruginosa* is not clear. This study aims to investigate the role of PMA in suppressing *P. aeruginosa* QS activation, and explore whether it could alleviate lung injury during early infection.

Material and methods

This study is comprised of experiments on *P. aeruginosa* culture, human airway epithelial cells stimulated with *P. aeruginosa*-culture filtrates, mouse models of *P. aeruginosa* lung infection, and bronchoalveolar lavage fluid (BALF) of bronchiectasis patients with first-time detection of *P. aeruginosa* infection. Figure 1 provides an overview of the study design and procedures.

Bacterial strains and PMA treatment

Wildtype *P. aeruginosa* PAO1 strains were grown in Lysogeny Broth (LB) medium with 50 mM 3-(*N*-morpholino) propanesulfonic acid at 37 °C with 250 rpm shaking overnight. When indicated, cultures were supplemented with different doses of PMA (0.01 mg/mL, 0.05 mg/mL, 0.5 mg/mL).

AHL and pyocyanin measurements

Signaling molecules were extracted using ethyl acetate, and both 3OC12-HSL and C4-HSL concentrations were measured using reporter strains as previously described [9, 10]. Pyocyanin was extracted from 4 mL culture fluid with 2 mL chloroform, and was then extracted from the chloroform with 1 mL 0.1 mol/L hydrochloric acid–water. At 520 nm, the absorbance was measured and multiplied by 17.072 to get the concentration of pyocyanin [11].

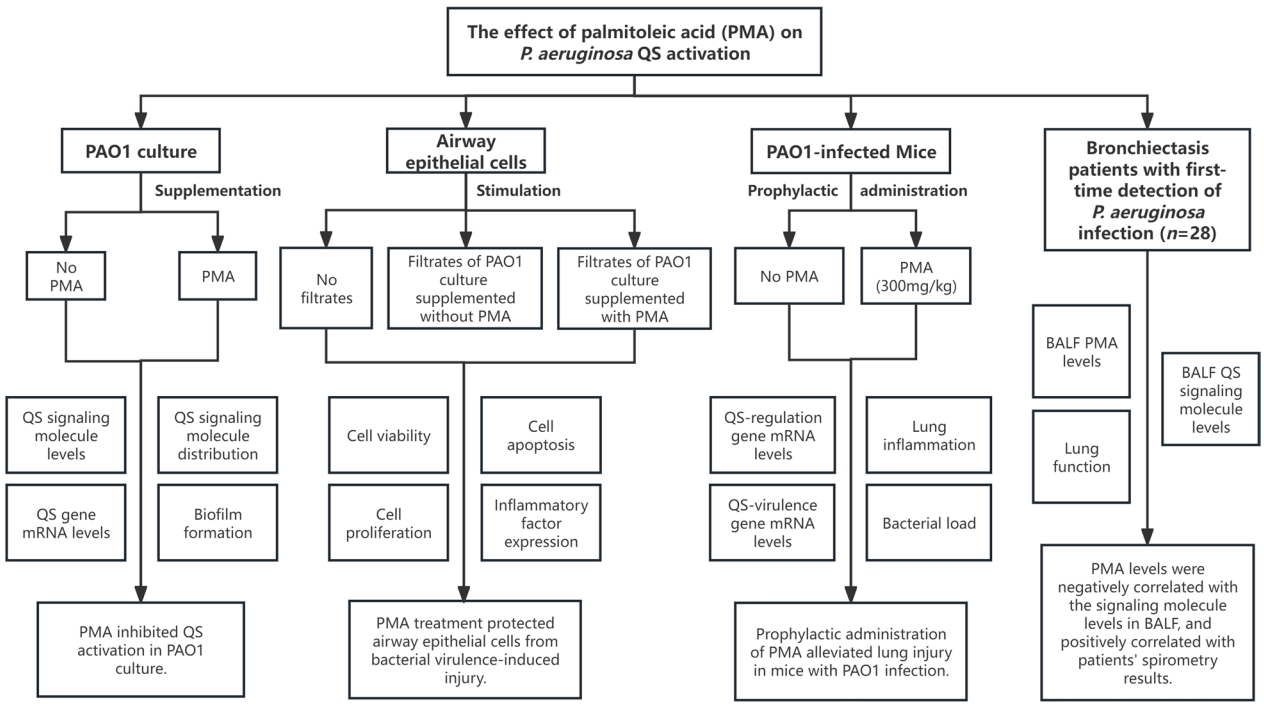

Fig. 1 Flow chart of the study design and procedures. *PMA* palmitoleic acid, *QS* quorum sensing, *BALF* bronchoalveolar lavage fluid

Biofilm detection

Bacterial biofilm was quantitatively detected using a 96-well microplate. Briefly, 100 μL LB medium with or without 0.5 mg/mL PMA was added to each well and incubated with 10 μL overnight PAO1 culture at 37 °C for 36 h. The plate wells were washed and fixed with methanol for 15 min. Add 100 μL 1% crystal violet solution to each well and dye at room temperature for 5 min, and then the wells were rinsed and dried. After complete drying, add 100 μL of 33% glacial acetic acid solution to each well and incubate at 37 °C for 30 min to dissolve crystal violet. Measure the optical density (OD) value of the solution in the culture well using a microplate reader (BioTek Epoch, BioTek Instruments, Winooski, VT, US) under 590 nm conditions.

Bronchial epithelial cell stimulation experiments

Pseudomonas aeruginosa filtrates were prepared as reported in a previous study [12]. Briefly, PAO1 cultures were grown in LB medium supplemented with PMA (0.5 mg/mL) for 24 h. Cultures grown in LB medium without PMA were used as controls. Cultures were centrifuged and filtered with 0.22-μm cellulose acetate filters, and the filtrates were collected. Human bronchial epithelial BEAS-2B cells (2.0×10^5 cells/well) were incubated in a starvation medium for 16 h, and then stimulated with 60 μL of sterile filtrates or LB medium for the time-points of 3 h, 6 h, 12 h, and 24 h.

Cell Counting Kit-8 kit (Signalway Antibody, Greenbelt, MD, USA) was utilized to assess cell viability. After stimulation, cells were incubated with 10 μL kit solution in 96-well plates for one hour. Optical density was measured at 450 nm. For cell apoptosis assay, cells were collected and stained with Annexin V-FITC (Beyotime Biotechnology, Shanghai, China). To analyse the cell cycle, cells were treated with propidium iodide (PI) and RNase A for 30 min. The proportion of cells in each phase was determined using the FlowJo software (FlowJo LLC, Ashland, OR, USA). Human interleukin (IL)-6, and IL-8 in the epithelial cell cultures were determined by the use of enzyme-linked immunosorbent assay kits (R&D Systems, Inc., Minneapolis, MN, USA). The mRNA levels of IL-6 and IL-8 were determined by qRT-PCR. Each experiment was performed in triplicate.

Prophylactic administration of PMA in mice with *P. aeruginosa* infection

Male C57BL/6 mice (specifically pathogen-free, 6 weeks of age) were purchased from SLAC (Shanghai, China). Experimental Animal Care and Ethics Committee of Shanghai General Hospital approved all experimental protocols used in this study. Mice were inoculated intratracheally with 50 μL *P. aeruginosa*-laden agarose beads (2.0×10^6 CFU/mL) as previously described [13]. Mice were prophylactically administered with PMA (300 mg/kg) by gavage daily starting 2 weeks before inoculation. Those administered with saline were used as controls. The treatment continued after inoculation until mice were euthanized at indicated times.

The lungs were lavaged, and the BALFs were collected and centrifuged at 3000 rpm for 10 min at 4 °C. BALF cells were counted using a hemocytometer by two independent observers. Lungs were aseptically homogenized in sterile saline for CFU enumeration, and lung myeloperoxidase (MPO) activities were measured by using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Hematoxylin/eosin-stained sections were blindly scored for inflammatory infiltrates using the scoring system [14].

RNA isolation and qRT-PCR

For PAO1 cultures, diluted cultures were grown at 37 °C, and when reaching the indicated OD_{600nm}, cells were pelleted and preserved in RNA Protect Bacteria reagent (Qiagen, Hilden, Germany). For mouse lung tissues, the left lungs were excised aseptically and kept in liquid nitrogen. Cell pellets or lung tissues were lysed in QIAzol Lysis Reagent. Then the total RNA was extracted and purified using the RNeasy MinElute cleanup kit (Qiagen, Hilden, Germany). The expression of target genes was analyzed by following the protocol for the iQ SYBR Green SuperMix (Bio-Rad Laboratories, Hercules, CA, USA) on a ViiA 7 PCR System (Applied Biosystems, CA, USA). *rplU* was used as the reference gene, and the primers used in the study are listed in Table S1.

P. aeruginosa QS signal measurements and spirometry tests in bronchiectasis patients

Bronchiectasis patients with first-time detection of *P. aeruginosa* infection were recruited between January 2020 to December 2023 from Shanghai General Hospital, China. At the time of admission, all patients presented with purulent sputum, and underwent spirometry tests, including forced vital capacity (FVC) and forced expiratory volume in the first second ($FEV_{1.0}$). Then the bronchoscopy examination was performed, and all patients were positive for *P. aeruginosa* ($>10^4$ CFU/mL) in the samples of BALF. The measurements of *P. aeruginosa* 3OC12-HSL and C4-HSL were performed using BALF samples as above described. The Medical Ethics Committee of Shanghai General Hospital approved the protocol.

PMA measurement in BALF

BALF sample mixed with chloroform methanol (2:1 v/v) had an ultrasonication of 30 min. Fatty-acid methyl

esterification was achieved by 30 min of 80 °C water bath and later mixed with n-hexane. The supernatant was subjected to an Agilent Model 7890A/5975C GC–MS system. An Agilent DB-WAX capillary GC column was used to separate the samples. PMA quantification (μg/mL) was performed by making a calibration curve using the Supelco 37-component FAME mix (Sigma-Aldrich). The stability and repeatability of the system was verified by using a quality-control sample.

Statistical analysis

Data were analyzed by GraphPad Prism (version 9; GraphPad Software, San Diego, CA, USA), and graphs were drawn accordingly. For normally distributed quantitative variables, *t*-test or variance was used for data analysis; while for non-normally distributed variables, Mann–Whitney U test was used. Correlation between two nonparametric variables was performed using Spearman correlation coefficient (*rs*). *P*-values less than 0.05 were considered statistically significant.

Results

PMA suppressed QS activation in PAO1 culture

We supplemented different doses of PMA to the PAO1 wildtype culture and performed time-course experiments. Compared with controls, the supplementation of PMA decreased the 3OC12-HSL and C4-HSL concentrations and reduced pyocyanin production in a dose-dependent manner (Fig. 2A–C). To further elucidate the role of PMA in inhibiting QS activation, we examined the impact of PMA (0.5 mg/mL) on mRNA levels of QS genes and observed that high-dose PMA down-regulated the mRNA levels of *lasR*, *rhlR*, and *rhlI* (Fig. 2D, E, G), while no marked effect on *lasI* mRNA was observed (Fig. 2F). The formation of biofilm was significantly decreased by PMA (Fig. 2H).

It's known that PMA is a major component in the bacterial membrane [15], so we examined whether PMA could alter membrane permeability to signaling molecules. In the wildtype culture, the ratios of intracellular/extracellular concentration for 3OC12-HSL were above 2.4 throughout the time course. When exogenous PMA (0.5 mg/mL) was supplemented, the ratios were significantly decreased to nearly 1.0 at the late logarithmic stage (Fig. 2I). While for C4-HSL, the ratio of intracellular/extracellular concentration was around 1.0 throughout the time course, and PMA didn't have a significant effect on C4-HSL distribution (Fig. 2J).

PMA treatment protected airway epithelial cells from bacterial virulence-induced injury

To test whether PMA would have a protective effect on airway epithelial cells, we stimulated immortalized wildtype BEAS-2B cells by bacteria-free filtrates that were collected using PAO1 culture treated with 0.5 mg/mL of PMA. We observed that when BEAS-2B cells were stimulated with PMA-treated filtrates, their viability was significantly higher than those stimulated with control filtrates (Fig. 3A). Further experiments indicated that PMA-treated filtrates significantly reduced cell apoptosis rates (Fig. 3B) and improved cell proliferation (Fig. 3C). The levels of both IL-8 and IL-6 (mRNA and protein expression) were significantly lower in cells stimulated with PMA-treated filtrates than those with control filtrates (Fig. 3D).

Prophylactic administration of PMA alleviated lung injury in mice with PAO1 infection

Having determined the protective role of PMA in alleviating virulence-induced epithelial cell injury, we set out to investigate whether prophylactic administration of PMA could relieve lung injury caused by *P. aeruginosa* infection. We administered mice with PMA (300 mg/kg) by gavage daily for 2 weeks and then inoculated mouse trachea with agarose beads laden with PAO1 wildtype strain. We observed that prophylactic administration of PMA not only downregulated the bacteria-derived mRNA expression of QS-regulation genes (*lasR*, *rhlR*, and *rhlI*), but also downregulated the mRNA expression of virulence genes (*lasB*, *rhlA*, *phzA1*, and *phnA*) on both Day 1 and Day 3 (Fig. 4A, B). Lung pathological injury (Fig. 4C, D), neutrophilic airway inflammation (Fig. 4E) as well as lung myeloperoxidase activity (Fig. 4F) were significantly reduced by PMA on Day 3. The bacterial load showed no significant difference between the two groups (Fig. 4G).

PMA levels in BALF were positively correlated with spirometry results of bronchiectasis patients with first-time detection of *P. aeruginosa* infection

We examined the levels of PMA and QS signaling molecules in the BALF samples from 28 bronchiectasis patients (56.2 ± 11.2 years old, 57.1% were female) with first-time detection of *P. aeruginosa* infection, and did spirometry tests for these patients. We found that BALF PMA levels were negatively correlated with BALF concentrations of QS signaling molecules (3OC12-HSL and C4-HSL) (Fig. 5A, B), and there were positive correlations between BALF PMA levels and the results of both FVC% and $FEV_{1.0}$% of predicted in these patients (Fig. 5C, D).

Discussion

The widespread use of antibiotics has led to the outbreak of antibiotic resistance. In the past 5 decades, the antimicrobial resistance rate of clinical *P. aeruginosa* isolates has sharply increased, bringing huge economic

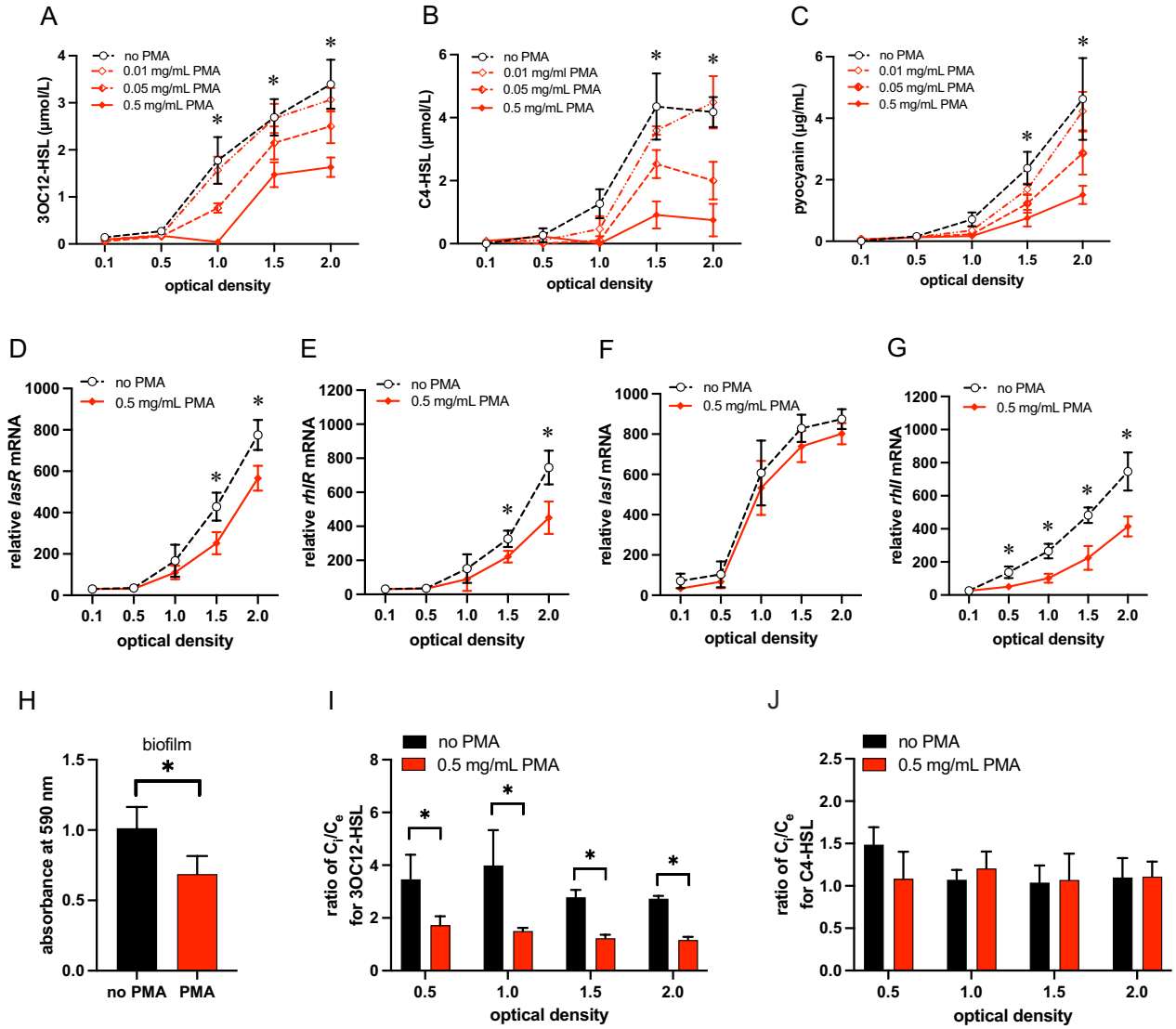

Fig. 2 Time-course experiments of phenotypes and quorum-sensing gene transcriptions for PAO1 wildtype cultures supplemented with palmitoleic acid. Total levels of 3OC12-HSL (**A**) and C4-HSL (**B**), as well as production of pyocyanin (**C**), were significantly decreased in the PAO1 culture by palmitoleic acid in a dose-dependent manner. High-dose palmitoleic acid down-regulated the mRNA levels of *lasR* (**D**), *rhlR* (**E**), and *rhlI* (**G**), while exhibiting no significant impact on the mRNA expression of *lasI* (**F**). Biofilm formation was significantly decreased by palmitoleic acid (**H**). The ratio of intracellular/extracellular 3OC12-HSL concentrations (C_i/C_e for 3OC12-HSL) was significantly decreased by high-dose palmitoleic acid (**I**), while the ratio of C4-HSL concentrations (C_i/C_e for C4-HSL) wasn't significantly affected (**J**). Data were presented as mean ± SD ($n = 3$). *$P < 0.05$ between indicated groups; *PMA* palmitoleic acid

and medical burdens to patients and healthcare systems [16]. The pathogenesis of *P. aeruginosa* is mostly due to an arsenal of secreted virulence factors regulated by QS systems, so anti-QS therapy is a promising strategy that targets *P. aeruginosa*'s pathogenicity rather than viability [17]. In contrast to conventional antibiotics that directly kill or inhibit bacterial growth, anti-QS therapy bypasses the evolutionary pressure on the bacterium to develop antimicrobial-resistance and super-infections, and thus might have potential therapeutic advantages over traditional antibiotics in the treatment of antimicrobial-resistant bacterial infections.

In this study, we found that PMA, a 16-carbon monounsaturated fatty acid, had the capability of suppressing the expression of QS signaling molecules and QS-dependent genes at the logarithmic growth stage in *P. aeruginosa*. Treating *P. aeruginosa* culture with PMA protected airway epithelial cells from bacterial virulence-induced injury. Prophylactically oral administration of PMA in mice infection models suppressed *P. aeruginosa*

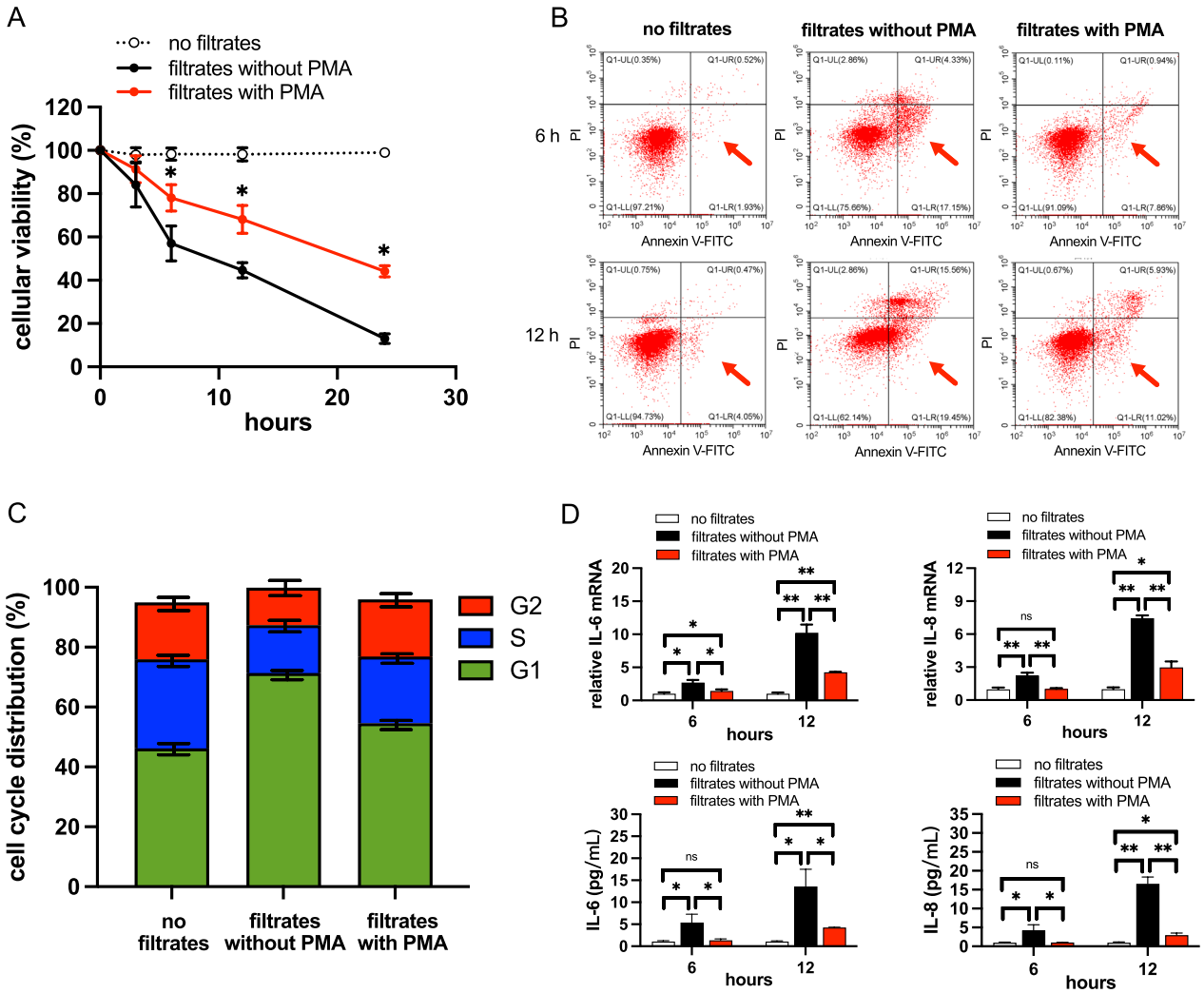

Fig. 3 Stimulating immortalized wildtype human bronchial epithelial BEAS-2B cells with *P. aeruginosa* filtrates. PAO1 wildtype cultures were treated with palmitoleic acid (0.5 mg/mL), and when their OD_{600nm} reached 2.0, they were filtered to get the bacteria-free filtrates. Cultures grown in LB medium without PMA were used as controls. When BEAS-2B cells were stimulated with palmitoleic acid-treated filtrates, their viability was significantly higher than those stimulated with control filtrates (**A**). The cell apoptosis (%Annexin V^+PI^- cells, red arrow, **B**) and cell proliferation disorder (**C**) were significantly improved by the treatment of palmitoleic acid. The mRNA and protein expressions of IL-8 and IL-6 were also significantly reduced in cells stimulated with palmitoleic acid-treated filtrates (**D**). Data were presented as mean ± SD ($n = 3$). *$P < 0.05$ between indicated groups; *PMA* palmitoleic acid

QS activation and relieved neutrophilic airway inflammation. In bronchiectasis patients with first-time detection of *P. aeruginosa* infection, BALF PMA levels were negatively correlated with BALF QS signaling molecule levels and positively correlated with patients' spirometry results. These data support that PMA could be potentially applied as an anti-QS agent to alleviate lung injury induced by *P. aeruginosa* infection.

Pseudomonas aeruginosa produces a variety of virulence factors, such as pyocyanin and protease, as well as forms biofilms in airways, which causes persistent neutrophilic inflammation and lung tissue injury [18]. QS system in *P. aeruginosa* primarily relies on the production and secretion of autoinducers as diffusible signaling molecules that activate their corresponding receptors when reaching a certain concentration threshold, thereby directly or indirectly regulating the production of virulence factors, enhancing biofilm formation, promoting immune evasion, and enabling bacterial colonization [7, 19, 20]. Therefore, suppressing QS function plays an important role in combating early *P. aeruginosa* infection. Our results showed that PMA has the capability of inhibiting *P. aeruginosa* QS activation from in vitro to in vivo, leading to reduced bacterial pyocyanin production and

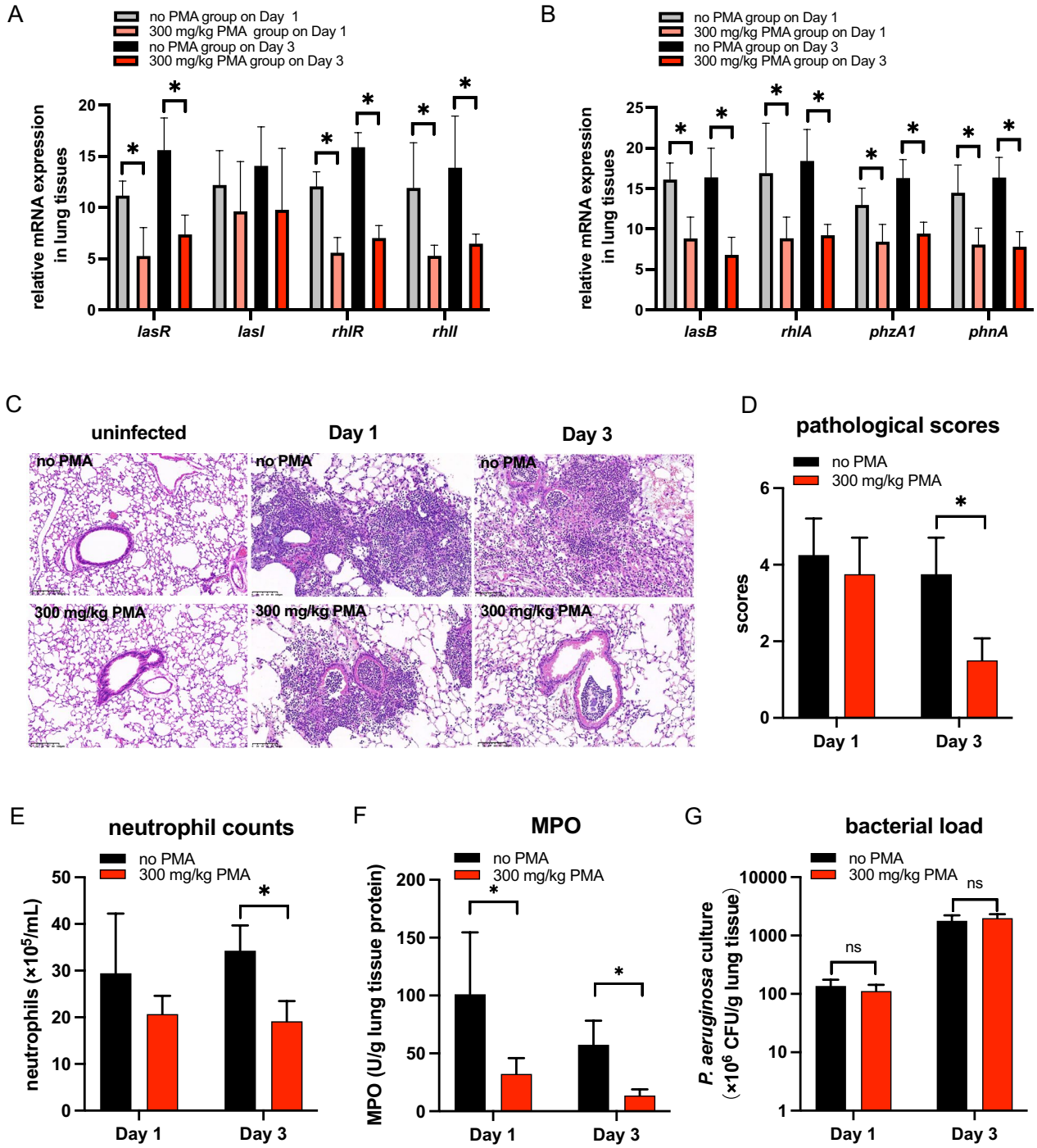

Fig. 4 *Pseudomonas aeruginosa* quorum sensing (QS) gene expression and infection-induced airway inflammation in mouse models after prophylactic administration of palmitoleic acid by gavage. Palmitoleic acid (300 mg/kg) or saline (control) was administered daily starting 2 weeks before inoculation of *P. aeruginosa*-laden agarose beads. On both Day 1 and Day 3, the expression of *P. aeruginosa* QS genes (*lasR*, *rhlR*, and *rhlI*), and QS-controlled virulence genes (*lasB*, *rhlA*, *phzA1*, and *phnA*) were significantly suppressed by palmitoleic acid (**A**, **B**). Consistent to the suppression of virulence, the pathological sections showed that lung injury caused by *P. aeruginosa* infection was relieved in palmitoleic acid-treated mice (**C**). The administration of palmitoleic acid improved lung pathological scores (**D**), and reduced bronchoalveolar lavage fluid neutrophil counts (**E**) and lung myeloperoxidase levels (**F**), but did not significantly affect bacterial load (**G**). Data were presented as mean ± SD ($n = 4$). *$P < 0.05$ between indicated groups; *PMA* palmitoleic acid, *MPO* myeloperoxidase

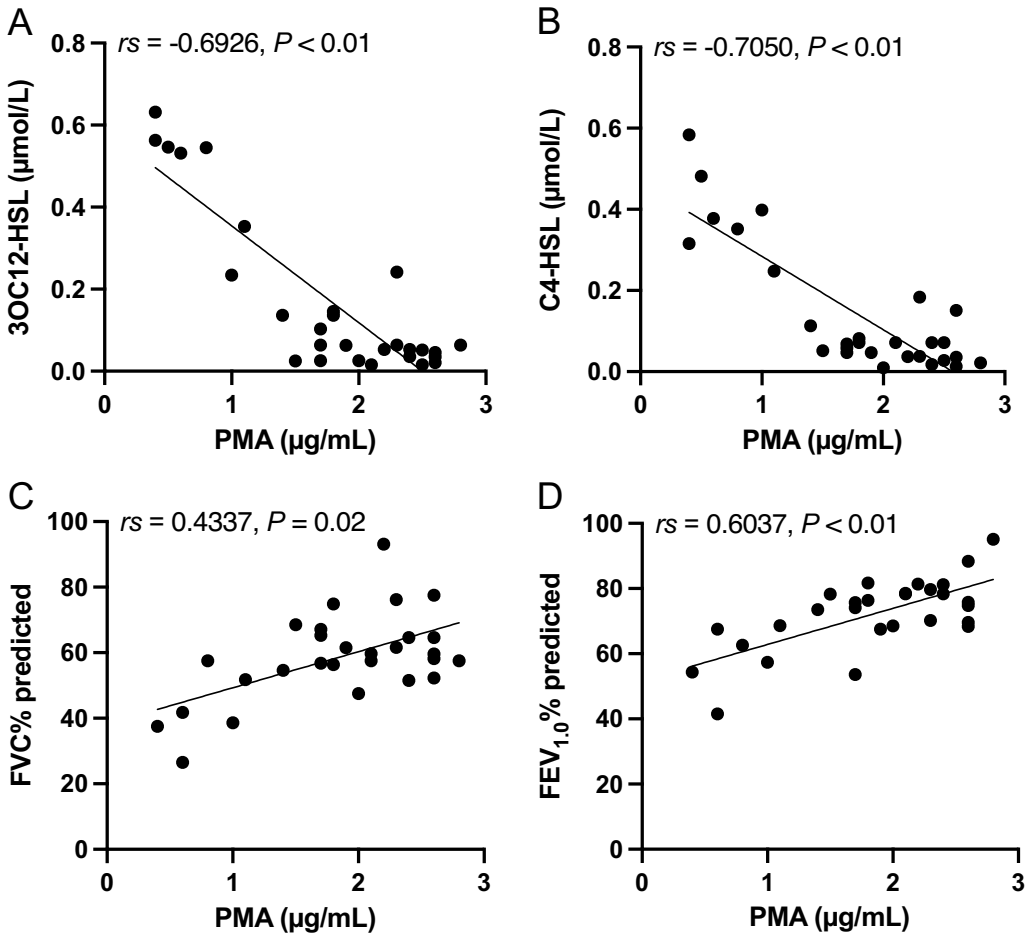

Fig. 5 Correlation analysis among palmitoleic acid levels, *P. aeruginosa* quorum sensing signal concentrations, and spirometry results in bronchiectasis patients with first-time detection of *P. aeruginosa* infection. The bronchoalveolar lavage fluids were collected, in which the PMA levels and *P. aeruginosa* quorum sensing signal concentrations were determined. PMA levels exhibited a negative correlation with the concentrations of signaling molecules 3OC12-HSL (**A**) and C4-HSL (**B**). Meanwhile, there were positive correlations between PMA levels and spirometry results (FVC% of predicted, **C**; $FEV_{1.0}$% of predicted, **D**) in these patients ($n = 28$). *PMA* palmitoleic acid, *FVC* forced vital capacity, $FEV_{1.0}$ forced expiratory volume in the first second; *rs* spearman's rank correlation coefficient

biofilm formation. These effects contributed to the alleviation of bronchial epithelial cell injury and lung damage caused by *P. aeruginosa* infection.

The application of anti-QS strategies needs to meet several requirements. Firstly, the expression of virulence factors should be mainly promoted by targeted QS systems, and interference with QS activation is capable of weakening the virulence [21, 22]. Secondly, interference with the QS system should not affect the growth of pathogens, thereby avoiding selective pressure and preventing the emergence of resistant strains [23]. Thirdly, the strategy of QS quenching is ideally deployed prior to pathogen proliferation reaching the threshold necessary to activate its pathogenicity [24]. In this study, we found that PMA suppressed the function of LasI-R and RhlI-R systems in *P. aeruginosa*, resulting in reduced production of pyocyanin and other soluble virulence factors, and thus alleviated bronchial epithelial cell injury. Furthermore, prophylactically oral administration of PMA suppressed *P. aeruginosa* QS-controlled virulence gene expression and relieved lung injury in mice. Whether in the culture medium or the host, PMA didn't have a significant effect on the bacterial load. These findings supported PMA as a candidate anti-QS agent for QS quenching strategies against *P. aeruginosa* infection.

The mechanism of PMA inhibiting QS activation might be related to the increased membrane permeability and enhanced 3OC12-HSL export, as it was observed that PMA reduced the ratio of *P. aeruginosa* cellular/extracellular 3OC12-HSL concentration rather than suppressed

the expression of *lasI* gene that encodes 3OC12-HSL synthetase. The maintenance of membrane homeostasis relies on the structural variety of saturated, monounsaturated, and polyunsaturated fatty acids. These components are crucial for precisely adjusting membrane fluidity and permeability properties [25]. PMA, which belongs to cis-unsaturated fatty acid, can increase membrane permeability and fluidity through the disruption of fatty acid tail packing [26]. Under normal physiological conditions, although most *N*-acyl homoserine lactone signaling molecules are assumed to diffuse in and out of cells freely, 3OC12-HSL has been proposed to be partitioned by membranes due to its long chain [27]. Our data showed that PMA downregulated the ratio of cellular/extracellular 3OC12-HSL concentration, suggesting it may increase bacterial membrane permeability to 3OC12-HSL, thereby decreasing cellular signal concentration and delaying QS activation. Although PMA may simultaneously act on bacterial pathways other than QS system, its suppressive effect on QS activation is one of the most important roles involved in weakening *P. aeruginosa* virulence.

PMA comes from the desaturation of palmitoyl CoA, a process that can stem from lipogenesis or directly obtained from the diet through the activity of D9 desaturase isomers [28]. Therefore, its level could increase when the endogenous lipogenesis is enhanced or the dietary intake of PMA is increased [29]. In human body, PMA has effects on fat synthesis and storage, intracellular trafficking, inter-organ signaling pathways, as well as cell differentiation and proliferation [30]. However, in lungs, its data on relieving infection-induced injury is scarce. In this study, we showed that PMA might inhibit *P. aeruginosa* QS activation in human lungs, as there was a negative correlation between PMA level and QS signal (3OC12-HSL, C4-HSL) concentrations in BALF from bronchiectasis patients with first-time detection of *P. aeruginosa*. Furthermore, we showed that PMA levels in BALF were positively correlated with the results of FVC% and $FEV_{1.0}$% predicted in these patients, supporting that PMA protected lung function during early infection with *P. aeruginosa*. While we cannot discount the possibility that PMA might exert other effects such as potential anti-inflammatory activities on host cells, its role in inhibiting bacterial QS activation likely constitutes one of the primary mechanisms that protect human lungs from infection-induced injury. From this perspective, increasing PMA intake in the diet could help prevent lung injury in patients with a high risk of *P. aeruginosa* infection.

In conclusion, our work showed that PMA inhibited *P. aeruginosa* QS activation and reduced QS-related virulence production. It protected human airway epithelial cells and mouse lungs from injury induced by *P. aeruginosa* virulence, and displayed the potential of improving lung function among bronchiectasis patients in the early stages of *P. aeruginosa* infection. Our data supported the anti-QS potential of PMA in tackling *P. aeruginosa* lung infection.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-024-03035-2.

Supplementary Material 1.

Acknowledgements
Not applicable.

Author contributions
F.D, L.H, J.R, and M.Z conceived of and designed the entire study. L.H, J.R, Y.X, G.X, J.G, Q.F, P.S, H.Z and F.D performed the experiments and statistical analyses. L.H and J.R wrote the manuscript, supervised by M.Z and F.D. All authors critically reviewed and approved the final version. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The animal study was reviewed and approved by the Experimental Animal Care and Ethics Committee of the Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine (IACUC: 2019-A011-01). All animal housing and experiments were conducted in strict accordance with the institutional guidelines for the care and use of laboratory animals. The studies involving human participants were reviewed and approved by the Ethics Committee of Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine (Approval No. 2020-50). The patients/participants provided their written informed consent to participate in this study. The study was performed in accordance with the 1964 declaration of HELSINKI and later amendments.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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