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Short communication

Varenicline is a smoking cessation drug that blocks alveolar expansion in mice intratracheally administrated porcine pancreatic elastase

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ABSTRACT

Smoking cessation is the most effective treatment in patients with emphysema and lung inflammation. The aim of the present study was to examine the effect of varenicline, a smoking cessation drug, on emphysema in porcine pancreatic elastase (PPE)-inhaled mice. PPE-inhaled mice were treated with varenicline and an α7 nicotinic acetylcholine receptor (nAChR) antagonist, methyllycaconitine (MLA) for 5 and 21 days. Varenicline markedly ameliorated alveolar expansion and inflammatory response in bronchoalveolar lavage fluid in PPE-inhaled mice. These blocking effects were inhibited by MLA. Our findings demonstrate that varenicline likely has an anti-inflammatory property including reduced inflammatory cell recruitment in lung tissue to protect PPE-induced alveolar expansion via α7 nAChR.

Chronic obstructive pulmonary disease (COPD) is characterized by progressive and irreversible limited airflow with emphysema and lung inflammation. COPD is estimated to become the third most common cause of death by 2020.[1] Acute-phase lung inflammation is known to be a major contributor to the development of emphysema.[2,3] Furthermore, inflammatory cells such as macrophages, neutrophils, and T cells play key roles in development and exacerbation of COPD.[4] Smoking is the most important risk factor for COPD, and therefore smoking cessation is the most effective treatment. Varenicline, a smoking cessation drug, is a selective α4β2 nicotinic acetylcholine receptor (nAChR) partial agonist and an α7 nAChR full agonist.[5,6]

It is more effective and frequently used clinically for smoking cessation.[7,8] Additionally, successful smoking cessation with varenicline reduces spirometric lung age.[9] However, it is unclear whether varenicline directly affects pulmonary diseases such as COPD, emphysema, and acute-phase lung inflammation. Here, we show that varenicline may protect against alveolar expansion via α7 nAChR in porcine pancreatic elastase (PPE)-inhaled mice.

All procedures involving experimental animals adhered to the Law (No. 105) and Notification (No. 6) of the Japanese Government, and were approved by the Laboratory Animal Care and Use Committee of Fukuoka University (Fukuoka, Japan). C57BL/6J mice (8-week-old, KBT Oriental Co., Saga) were anesthetized by isoflurane inhalation (Wako, Osaka, Japan). Next, 2 U of PPE (Sigma–Aldrich, Saint Louis, MO, USA) were dissolved in 50 μL sterile phosphate-buffered saline (PBS) or 50 μL PBS alone were sprayed into the trachea using a MicroSprayer aerosolizer (Penn-Century Inc., Philadelphia, PA, USA), as previously described.[10] PPE-inhaled mice were subcutaneously injected with saline (vehicle group) or varenicline (0.5 mg kg^{-1} day^{-1}) (Toronto Research Chemicals Inc. Toronto, ON, Canada) and injected with methyllycaconitine (MLA) (5 mg kg^{-1} day^{-1}, i.p.) (Sigma–Aldrich) in combination with varenicline (0.5 mg kg^{-1} day^{-1}, s.c.) for a 5-day and 21-day period starting at 1-day post-inhalation. Mice were sacrificed under anesthesia. Then, lung tissue was collected from each mouse after perfusion with PBS and 4% formalin through the left cardiac ventricle. Lung sections (7 μm thick) were stained with hematoxylin and eosin. Lung images were obtained using a Keyence BZ-X710 microscope (Keyence Corporation, Osaka), and the distance between alveolar walls in randomly selected areas was measured using Image J software and was calculated from five non-overlapping microscopic fields per section from three different

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sections (15 fields) obtained from each mouse, as previously described.[10]

After treatment with varenicline for 5 days, bronchoalveolar lavage fluid (BALF) collected with PBS (0.5 mL × 4) was centrifuged at 1000 × *g* for 10 min at 4 °C, and cell pellets were resuspended in PBS (200 μL). Macrophages, neutrophils, and T cells in BALF were identified using anti-F4/80 antibody (BMA Biomedicals, Augst, Switzerland), anti-NIMP-R14 and anti-CD4 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA), respectively. Alexa Fluor 594-conjugated antibody (Molecular Probes, Carlsbad, CA, USA) was used as the secondary antibody. Cell numbers were counted using a microscope.

All results are expressed as mean ± standard deviation (SD). Statistical comparisons among three and four groups were performed by analysis of variance followed by Tukey's multiple comparison tests. Values of $P < 0.05$ were considered statistically significant.

First, the effect of varenicline against PPE-induced alveolar expansion was examined (Fig. 1A). At 3-weeks post-inhalation, average mean linear intercept (MLI) values were 52.56 ± 6.13 μm and 103.07 ± 23.65 μm in 0 U and 2 U PPE-inhaled mice, respectively (Fig. 2). When started at 1-day post-inhalation, varenicline treatment (0.5 mg kg^{-1} day^{-1} for 21 days) markedly suppressed PPE-induced alveolar expansion by 38% compared with controls ($P < 0.01$).

To determine the mechanism by which varenicline suppresses PPE-induced alveolar expansion, we examine the effect of MLA (5 mg kg^{-1} day^{-1} for 21 days), an α7 nAChR antagonist, on

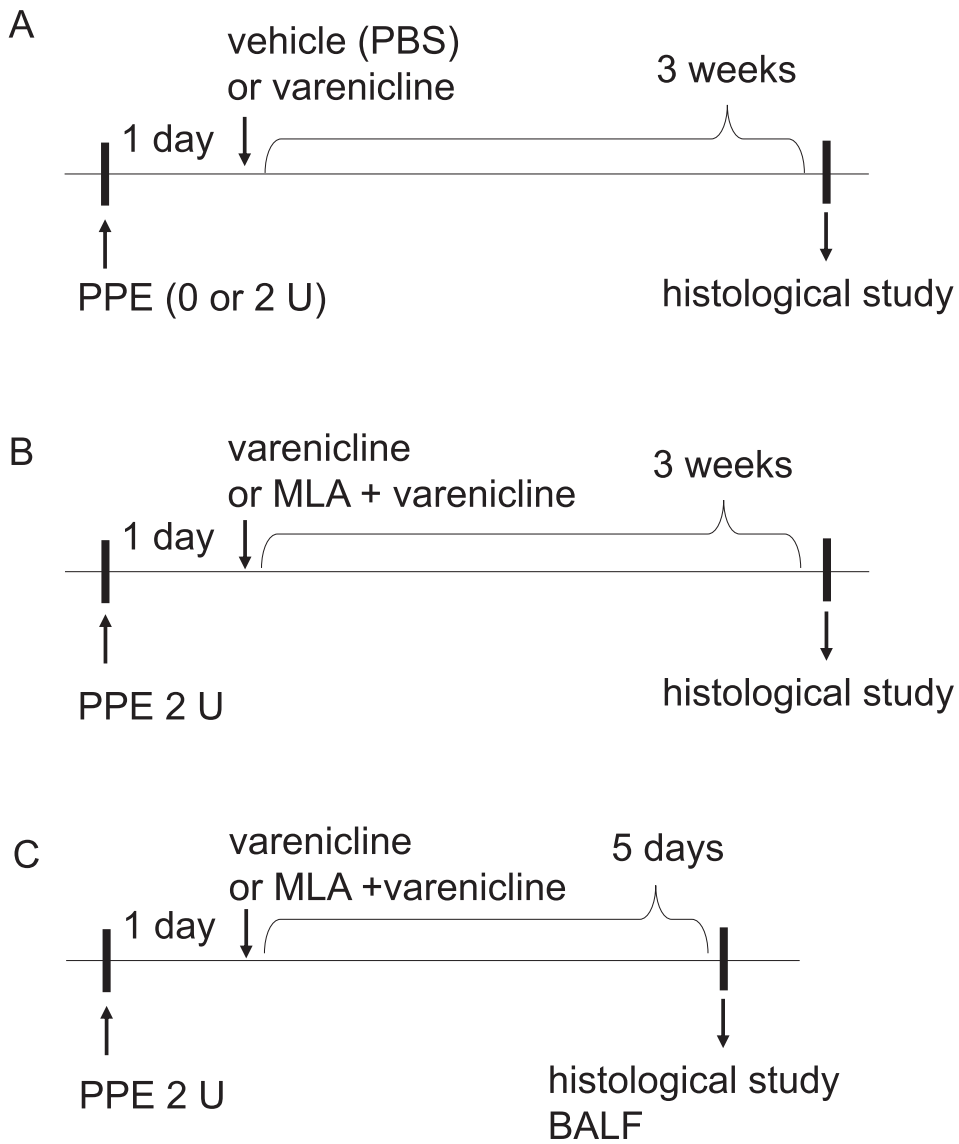

Fig. 1. Experimental protocols. (A) Effect of varenicline treatment for a 21-day period on alveolar expansion in PPE-inhaled mice. (B) Effect of MLA treatment for a 21-day period on varenicline-increased alveolar expansion in PPE-inhaled mice. (C) Effect of varenicline treatment for a 5-day period on inflammatory cell number in BALF and alveolar expansion in PPE-inhaled mice.

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