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Short communication

Elevated permeability of the blood–brain barrier in mice intratracheally administered porcine pancreatic elastase

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) shows progressive, irreversible airflow limitation induced by emphysema and lung inflammation. The aim of the present study was to determine if COPD conditions induce blood–brain barrier (BBB) dysfunction. We found that the intratracheal administration of porcine pancreatic elastase (PPE; 3 U) induced alveolar enlargement, increased neutrophil number in bronchoalveolar lavage fluid, and decreased blood oxygen saturation in mice at 21 days after inhalation. In parallel with these lung damages, BBB permeability to sodium fluorescein and Evans blue albumin was markedly increased. Our findings demonstrate that COPD conditions are associated with risk for BBB impairment.

Chronic obstructive pulmonary disease (COPD) shows progressive and irreversible airflow limitation with emphysema and lung inflammation. In addition to a systemic inflammatory component, neuropsychiatric symptoms including anxiety, depression, and cognitive impairment occur in COPD patients (1). The blood–brain barrier (BBB), an interface between the central nervous system and peripheral blood circulation, maintains brain milieu and limits entry of blood-derived neurotoxic proteins including therapeutic drugs (2). Impairment of the BBB occurs under several peripheral inflammatory diseases and allows transfer of BBB-impermeable drugs into the brain, causing drug-associated neurotoxicity (3). Dysregulation of BBB integrity precedes the onset of neurodegeneration, suggesting that brain damage develops in association with BBB impairment. Under these conditions, the brain is considered to become vulnerable to the central adverse reactions of drugs (2,4). COPD patients are prescribed muscarinic antagonists, β2-agonists, and phosphodiesterase-4 inhibitors to improve airflow obstruction, and varenicline for smoking cessation (5,6). In addition, they often require several drugs for other diseases, particularly elderly COPD patients. Thus, COPD patients are at high risk of central adverse drug reactions. For example, inhaled β2-agonists can cause headaches, tremors, and agitation (7). However, COPD-induced BBB alterations have remained obscure. Here, to clarify BBB pathophysiology under COPD conditions, we generated a COPD mouse model by intratracheal administration (i.t.) of porcine pancreatic elastase (PPE) according to the method previously reported by Kurimoto et al. (8) and subsequently examined BBB permeability in COPD 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. C57BL/6 mice (Clea Japan Inc., Tokyo, Japan), aged 7 weeks, were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg i.p.), followed by a spray of 0.3 or 3 U PPE (Sigma, St. Louis, MO, USA) dissolved in 25 μL sterile phosphate-buffered saline (PBS) or 25 μL PBS alone (vehicle group) into the trachea using a MicroSprayer drug delivery device (Penn-Century Inc., Philadelphia, PA, USA). Twenty-one days after PPE or PBS i.t., mice were sacrificed to harvest tissue under anesthesia.

Lungs were perfused with PBS (0.4 mL × 4) and bronchoalveolar lavage fluid (BALF) was collected from each mouse. BALF was

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immediately centrifuged at 1000 ×*g* for 10 min at 4 °C, and after lysing red blood cells in Red Blood Cell Lysing Buffer (Sigma), the cell pellets were resuspended in PBS (100 μL). Neutrophils in BALF were identified by Giemsa staining, and cell numbers were counted under a standard light microscope. After perfusion with PBS and 8% formalin through the left cardiac ventricle, lung tissue was collected from the superior lobe of the left lung in each mouse and stored in formalin for 48 h. Serial sections (7 μm thick) of the lung were prepared and stained with hematoxylin and eosin (H&E). Lung images were obtained using a Keyence BZ-X710 microscope (Keyence Corporation, Osaka, Japan), and the distance between alveolar walls in randomly selected areas was measured using ImageJ software (http://rsb.info.nih.gov/). The averaged linear intercept of alveoli was obtained from 3 or 4 non-overlapping microscopic fields per section in three different sections (10 fields) from each mouse. Oxygen saturation (SpO2) was recorded using a pulse oximeter under anesthesia (PowerOxy900; Bio Research Center, Nagoya, Japan).

An *in vivo* BBB permeability test was performed as described previously (9). In brief, PBS (150 μL) containing sodium fluorescein (Na-F) (2 mg/mL; Sigma) and Evans blue (EB) (20 mg/mL; Sigma) was injected into the jugular vein under anesthesia. Thirty minutes after intravenous injection, blood was collected from the right atrium and centrifuged at 1000 ×*g* for 10 min at 4 °C. Mice were perfused with PBS and then decapitated. The whole brain was removed and weighed. Brains and serum were homogenized in 0.5 M borate buffer (pH 10) and centrifuged at 800 ×*g* for 15 min at 4 °C. Supernatants were mixed with ethanol and then centrifuged (15,000 ×*g*) for 20 min at 4 °C. Supernatant Na-F and EB concentrations were determined as described previously (10). The brain/serum ratio was calculated according to the method described by Abraham et al. (11), and was expressed as microliters of tracer diffusing from the blood to brain per grams of brain.

Graph Pad Prism 5.0 (GraphPad, San Diego, CA, USA) was used to perform all statistical analyses. Results are shown as means ± S.E.M. The Student's t-test was used for comparison of neutrophil numbers and SpO2. Other data were assessed by one-way analysis of variance, followed by Tukey–Kramer's test for multiple comparisons. Differences were considered significant with P values <0.05.

As shown in Fig. 1A, H&E-stained histological sections revealed normal lung structure in the vehicle group. Moreover, no pathological changes were observed between vehicle and 0.3 U PPE treatment. However, with 3 U PPE inhalation, mice developed significant alveolar enlargement compared with vehicle- and 0.3 U PPE-treated mice (n = 5 mice per group, $P < 0.05$ versus vehicle or 0.3 U PPE) (Fig. 1B). The neutrophil number in BALF at 21 days after PPE inhalation was determined, with 5481 ± 939 and 8757 ± 1200 cells/mL in vehicle- and 3 U PPE-inhaled mice (60% increase), respectively (n = 7–8 mice per group, $P < 0.05$ versus vehicle). SpO2 significantly decreased with 3 U PPE to 93.5 ± 1.5% of vehicle at 21 days after inhalation (n = 10–11 mice per group, $P < 0.05$ versus vehicle) (Fig. 1C). PPE at 3 U (but not 0.3 U) significantly increased BBB permeability for Na-F (MW 376) and EB albumin (EBA) (MW 67,000) in mouse brain (28.2 ± 6.7 and 54.8 ± 16.0% increase, respectively) (n = 10–19 mice per group, $P < 0.05$ versus vehicle) (Fig. 2).

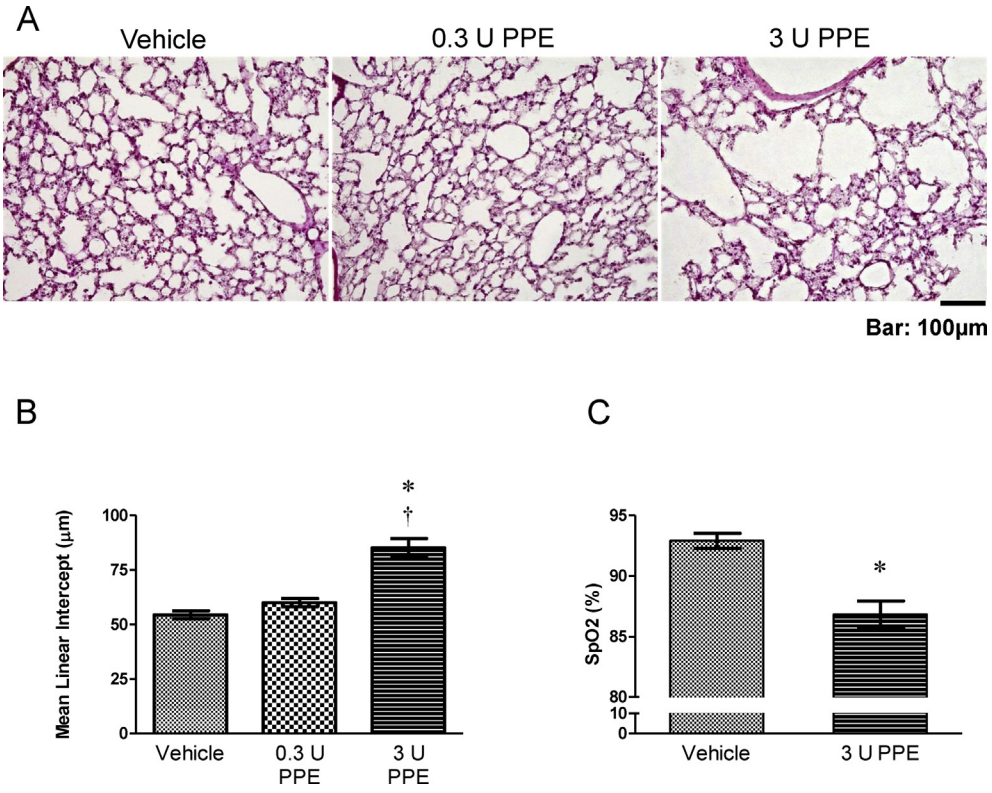

Fig. 1. PPE inhalation-induced damage to lung morphology and function. Histological observation of lung tissue and blood gas analysis were performed at 21 days after PPE i.t. (A) Representative photographs show H&E-stained lung sections at 21 days after vehicle, 0.3 U PPE, and 3 U PPE inhalation (n = 5 mice per group). (B) PPE-induced alveolar enlargement at 21 days after inhalation. The mean linear intercept of alveoli in H&E-stained lung sections was calculated in each mouse. Each bar indicates mean ± S.E.M. (C) Arterial oxygen saturation at 21 days after vehicle and 3 U PPE inhalation (n = 10–11 mice per group). Data are expressed as mean ± S.E.M. *$P < 0.05$, significantly different from vehicle. †$P < 0.05$, significantly different from 0.3 U PPE.