Aerosol Treatment with a Novel Inhibitor of Soluble Epoxide Hydrolase in a Murine Model of Asthma

XY Li [1,2], CZ Zhang [1,3], K Tsuji [4], J Yang [5], JJ Zhang [1], J Chen [1], CW Wu [1], SM Mack [1], W Li [2], M Takeuchi [4], KE Pinkerton [1], BD Hammock [5]

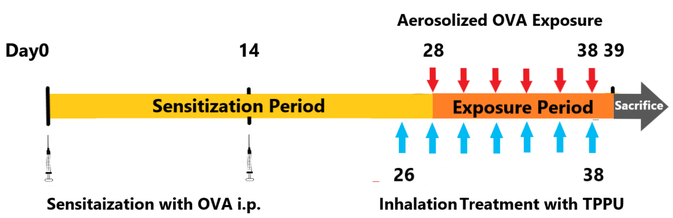
[1] Center for Health and the Environment, University of California, Davis - CA/US, [2] Shandong University - Jinan/CN, [3] Shandong Provincial Qianfoshan Hospital – Jinan/CN, [4] Kyoto Sangyo University – Kyoto/JP, , [5] Department of Entomology and Nematology and Comprehensive Cancer Center, University of California, Davis - CA/US

Introduction

Asthma affected around 339 million people worldwide, and further 100 million people will be likely to suffer by 2025. To achieve good asthma control, there are various guidelines for the management of asthma, but there is no radical or effective cure. Currently, targeting to soluble epoxide hydrolase (sEH) becomes a hot topic as a novel approach to many diseases, eg. lung inflammation, chronic obstructive pulmonary disease. Moreover, inhibitor of sEH might play roles in anti-inflammation, relaxing airways and anti-hypertrophy. We hypothesized that, 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU) could attenuate asthma by reducing airway inflammation, airway hyper-responsivity (AHR) and remodeling. In this study, ovalbumin (OVA) challenge murine model was used to investigate whether administration of TPPU by inhalation could dampen airway inflammation, constriction as well as mucin hypersecretion.

Materials & Methods

A total of four treatment groups were tested : 1) PBS control, 2) OVA, 3) OVA+TPPU (2h), 4) OVA+TPPU (6h). Male BALB/c mice were given 1% ovalbumin (OVA) by intraperitoneal (i.p.) injection on Day 0 and Day 14 to be sensitized, then Nebulized OVA (1%) for 1 hour was subsequently administered six times, every other day beginning on Day 28, to provoke allergic inflammation, AHR and mucin hypersecretion. Two groups of mice were pretreated with inhalation of 1 mg/ml TPPU for 2 or 6 hours on Day 26, then on alternate days, respectively. Pulmonary function test (PFT) using a FlexiVent system measured airway resistance. Lung tissues were collected to assess lung inflammation by a semi-quantitative scoring based on Hematoxylin and Eosin (H&E) staining. The value of airway constriction severity and the ratio of constricted airways to total airways were multiplied together to create the definitive score of airway contraction severity. Then ImageJ software was used to quantify mucin volume per surface area after alcian blue/periodic acid-Schiff (AB/PAS) staining. The complete exposure design is illustrated below.



Acknowledgements

Support for this research has been provided through funding from the American Asthma Foundation.

Results

Pulmonary Function Test

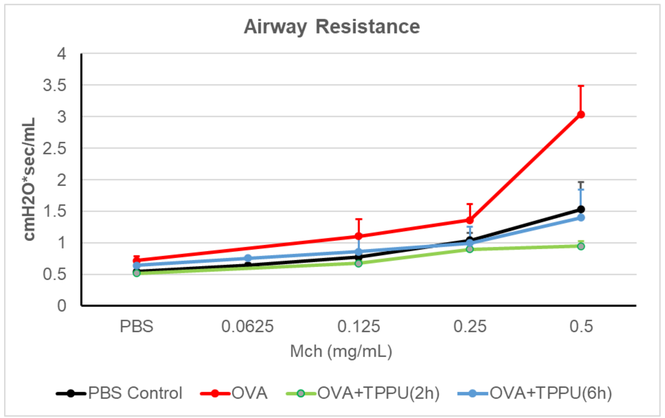


Figure 1. PFT was performed with increasing doses of methacholine (Mch). Compared to OVA group, mice with inhalation of TPPU for 2h, significantly reduced the airway resistance (P <0.01), when they were administrated with 0.5mg/ml Mch. Meanwhile, OVA+TPPU (6h) group showed a similar degree with control group. It indicated that TPPU inhalation had a possible trend to reduce AHR induced by OVA.

Histopathology

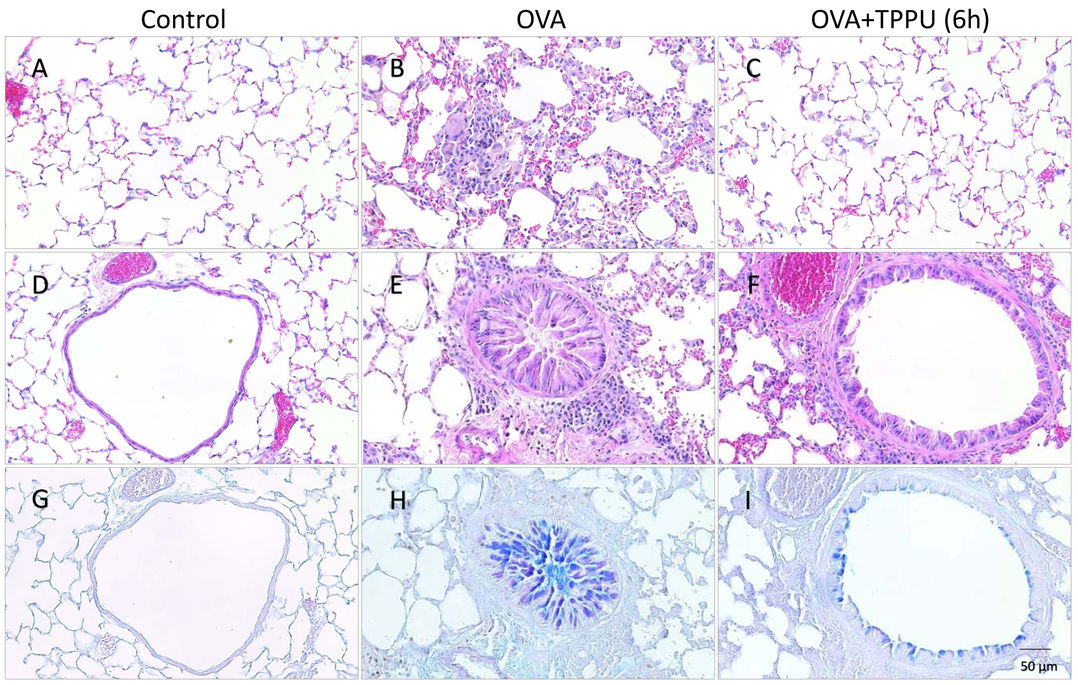


Figure 2. Light micrographs of lung tissue show inhalation of TPPU significantly attenuated alveolar inflammation caused by OVA based on H&E staining (A-C), as well as bronchial inflammation (D-F). D-F and G-I depict that inhaling TPPU apparently alleviated airway constriction severity of mice exposed to OVA, in addition to mucin volume per surface area by AB-PAS staining (G-I).

Results

Inflammation Scoring

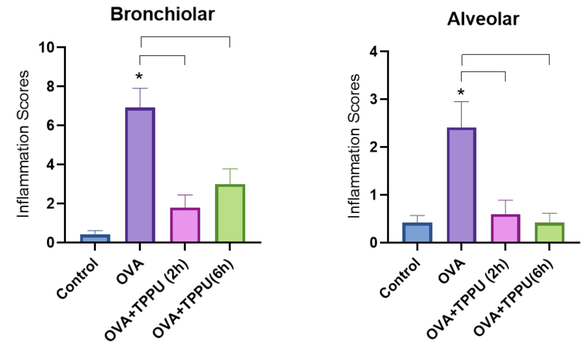


Figure 3. Inflammation in bronchiolar and alveolar regions of lung were scored, based on H&E staining. No matter in bronchioles or alveoli, TPPU treatment groups of 2h and 6h had an apparent reduction by 2-4 times in inflammatory response compared to OVA group, with statistical significances (P<0.05). It is easy to speculate that inhalation of TPPU significantly relieved lung inflammation in murine models after exposure to OVA.

Airway Morphology

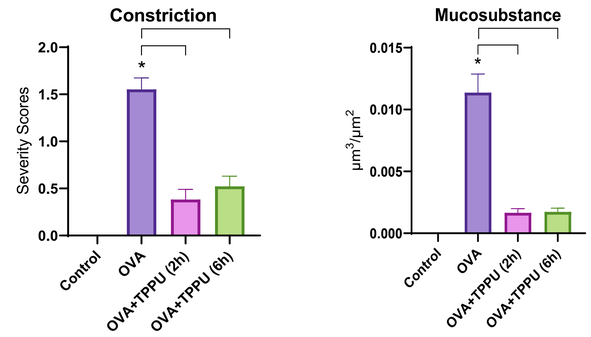


Figure 4. Morphometric and semi-quantitative measurements demonstrated that both of airway constriction severity and intracellular mucosubstance volume per basal lamina surface were decreased significantly (P <0.01) followed by inhalation of TPPU for either 2 or 6h, in comparation with exposure to OVA only. It suggests that TPPU given by inhalation directly reduced AHR and mucin hypersecretion in murine model of asthma.

Conclusions

Our study demonstrated that TPPU could ameliorate asthma through reduction of inflammation, suppression of AHR and airway epithelial remodeling. It provided a novel and prospective therapy for asthma in the future.