Appendix 1

Supplementary materials and methods

Animals and experimental design

A total of 10 C57BL/6J mice (male, 6–8 weeks, 20–25 g) were used in the pre-experiment after random allocation to the following groups: (I) control group (PBS): PBS (same volume as that in the LPS group) was injected into the trachea; (II) LPS group (LPS): different concentrations of LPS were injected into the trachea of mice according to their weight (5 and 10 mg/kg); stimulation was allowed to continue for 6 or 24 h.

Supplementary results

PKCα phosphorylation is upregulated in LPS-treated mice

The pre-experiment showed that lung injury was the most obvious after the intratracheal infusion of LPS (10 mg/kg) for 24 h (*Figure S1A*). Therefore, we used this dose and stimulation duration to establish an LPS-induced ALI model. Earlier research has shown that PKC isoform activity is altered in the event of ALI. In the pre-experiment, the activity of PKCα in mouse lung tissue was detected using Western blots. We found that LPS upregulated p-PKCα/PKCα expression irrespective of concentration (*Figure S1B*). Hence, we hypothesized that PKCα may participate in the pathogenesis of ALI. To test this hypothesis, we used Calphostin C, an inhibitor of PKCα, for subsequent experiments.

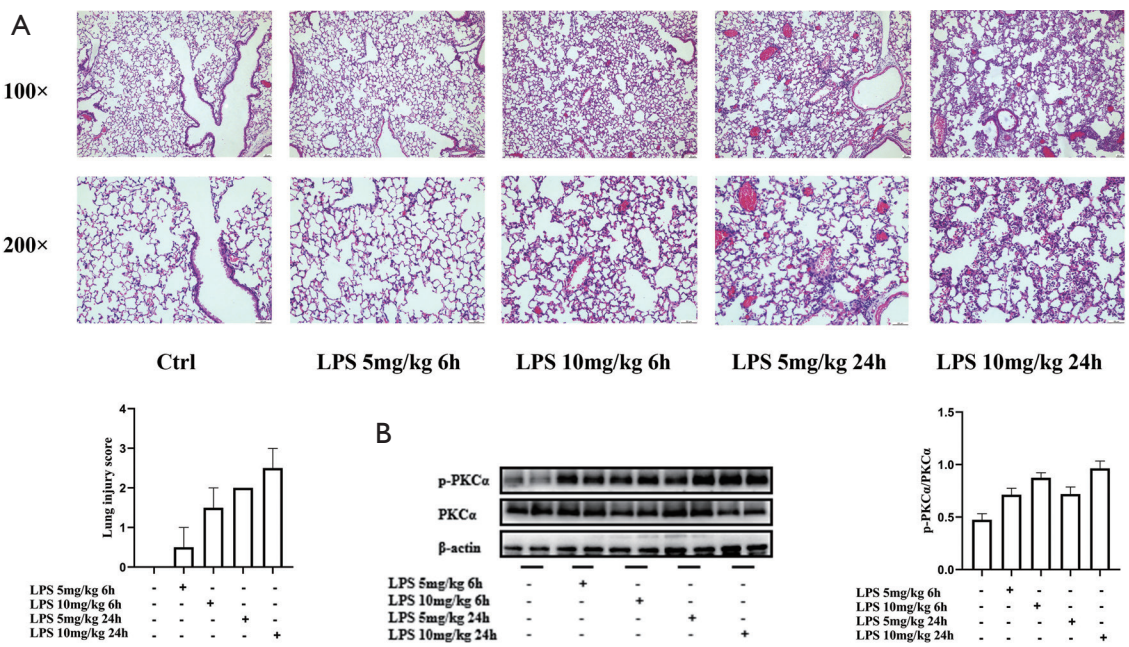

Figure S1 PKCα phosphorylation was up-regulated in LPS-triggered ALI mice. (A) Histopathological analyses of lung tissue (H&E staining; ×100 and ×200). (B) Protein levels of PKCα in lung tissue. "–" stands for the corresponding substance is not used; "+" stands for the corresponding substance is used. PKCα, protein kinase C alpha; ALI, acute lung injury; H&E, hematoxylin and eosin.

https://dx.doi.org/10.21037/atm-21-6497