

Supplementary materials and methods

A total of 30 adult male Sprague-Dawley (SD) rats (8-10 weeks; 220-270 g) were provided by Shanghai SLAC Laboratory Animal Co., Ltd. [SYXK (Shanghai) 2018-0038]. Rats were placed in a clean environment (20°C; 50-60% humidity) and raised in a 12-h light/dark cycle. All animals were euthanized by an intraperitoneal injection of pentobarbital sodium (≥ 100 mg/kg).

SD rats were assigned to 5 groups (6 rats in each group): Acute lung injury (ALI) + saline group, ALI + olive oil group and 2, 5, 8 mg/kg + all-trans retinoic acid (ATRA) groups. Rats in the ALI + saline group and ALI + olive oil group were injected with 5 mg/kg lipopolysaccharide (LPS) intravenously. Rats in ALI + saline group were administered saline (0.5 ml/kg/time) or olive oil (0.5 ml/kg/times) by gavage daily for 5 days before LPS injection. Following LPS injection, rats in ALI + saline group were intraperitoneally injected with saline (1 ml/kg) or olive oil (1 ml/kg). Then, five days before LPS injection, rats in 2, 5, 8 mg/kg + ATRA groups were intraperitoneally injected with olive oil containing 30 mg/kg ATRA (0.5 ml/kg/time). Following LPS injection, rats were intraperitoneally injected with olive oil containing 2, 5 and 8 mg/kg ATRA (1 ml/kg) for 7 consecutive days. On day 7, 24 h following an intraperitoneal injection of ATRA, rats in each group were euthanized and sampled. Detection of arterial PaO₂, lung wet/dry (W/D) weight ratio, protein content in bronchial alveolar lavage (BALF) fluid and hematoxylin and eosin (HE) staining were the same as in the main text.

Supplementary results

The rat model of ALI was established by LPS injection. The arterial blood and BALF of rats in each group were collected at 24 h after modeling to evaluate the effects of different concentrations of ATRA on the model rats. PaO₂ concentration and protein content were measured and the lung W/D ratio was determined. Compared with those in the 5 mg/kg ATRA group, the rats in the 2 and 8 mg/kg ATRA groups showed significantly reduced PaO₂ concentration and notably increased protein content and lung W/D ratio (Fig. S1A-C). HE staining was used to measure the pathological change of lung tissues and the lung injury score. Compared with those in the 5 mg/kg ATRA group, the rats in the 2 and 8 mg/kg ATRA groups showed significantly increased inflammatory cells in alveoli, dilated and congested pulmonary interstitial capillaries and elevated lung injury score (Fig. S1D and E). These results indicated that 5 mg/kg ATRA had an improved protective effect on ALI rats.

The rat model of ALI was established by LPS injection. The arterial blood and BALF of rats in the ALI + saline and ALI + olive groups were collected at 24 h after modeling to evaluate the effects of saline and olive on the model rats. PaO₂ concentration and protein content were measured and the lung W/D ratio was determined. The results showed that there was no significant difference in PaO₂ concentration, protein content and W/D ratio between the ALI + olive and ALI + saline groups (Fig. S2A-C). HE staining showed that there was no significant difference between the two groups in lung pathological change and lung injury score (Fig. S2D and E). These results indicated that olive oil had no significant effect on the model rats.

Figure S1. Index detection of rats in the ALI + olive oil group and ALI + saline group. (A) Protein content in BALF. (B) PaO₂ concentration in arterial blood. (C) Lung W/D ratio. (D) Lung injury in rats observed using hematoxylin and eosin staining. Magnification, x200. (E) Lung injury score, higher score indicated more serious injury. Data are expressed as mean ± standard deviation and analyzed using one-way analysis of variance, followed by Tukey's multiple comparisons test. N=6. ATRA, all-trans retinoic acid; ALI, acute lung injury; BALF, bronchial alveolar lavage fluid; PaO₂, arterial partial pressure of oxygen; W/D, wet/dry weight.

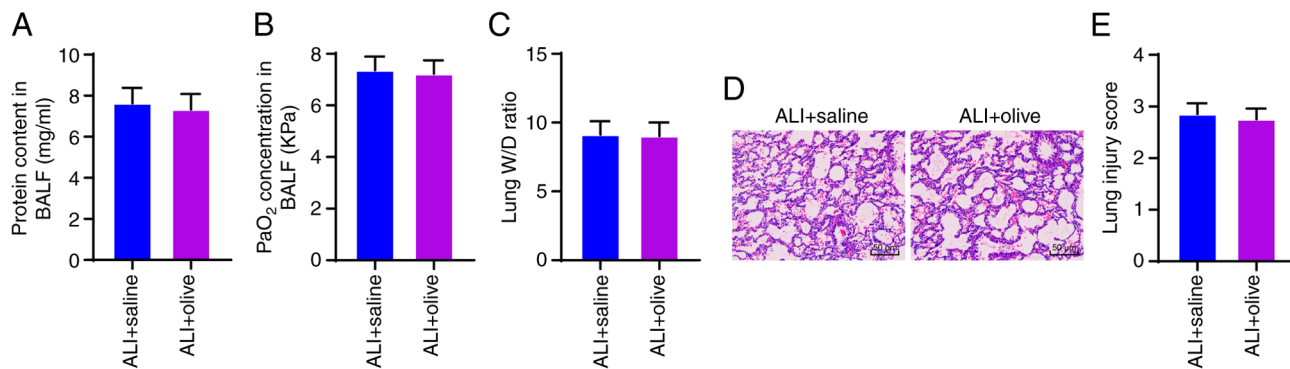


Figure S2. ATRA at 5 mg/kg possesses an improved protective effect on ALI rats. (A) Protein content in BALF. (B) PaO₂ concentration in arterial blood. (C) Lung W/D. (D) Lung injury in rats observed using hematoxylin and eosin staining. Magnification, x200. (E) lung injury score, higher score indicated more serious injury. Data are expressed as mean ± standard deviation and analyzed using the t-test. *P<0.05. N=6. ALI, acute lung injury; BALF, bronchial alveolar lavage fluid; PaO₂, arterial partial pressure of oxygen; W/D, wet/dry weight.

