Supporting Information

Antioxidative and Anti-inflammatory Activities of Paeoniflorin and Oxypaeoniflora on AGEs-Induced Mesangial Cell Damage

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1. Preparation for AGEs

The final AGEs can be obtained according to the following preparation method. Namely, 5 g of BSA and 9 g of D-glucose were weighed accurately and dissolved in 100 mL phosphate buffer saline (0.2 M, pH=7.4). The solution was passed through 0.22 μm microporous membrane to avoid the contamination and then incubated at 37 °C for 12 weeks under sterile conditions. The brown reaction solution was dialyzed overnight in 0.1 M PBS to remove these free small molecules. Finally, the obtained AGEs were identified by the specific fluorescence wavelength at excitation/emission = 370 nm/440 nm and quantified by AGEs ELISA kit. The AGEs were stored at 4 °C for further experiments.

2. Cells administered

Rat mesangial cells were maintained in low-glucose DMEM medium containing 10% fetal bovine serum (FBS) in 95% air/5% CO₂ and incubated in an incubator at 37 °C. The entire medium should be replaced every 2 days. After being seeded into 24-well plates, the cells were incubated for 24 h until growing to 80%. The cells were treated with Pae and Oxypae and followed with 200 μg/mL AGEs. Equal concentration of BSA (200 μg/mL) was used for the blank control. VE (μM) and AG (μM) were chosen for the positive drugs. After incubation for 24 h, the cell supernatant or cells were used for experiments.

3. Migration of macrophages

The migration of macrophages experiment was performed in accordance with the method in our previous studies. All the male mice about 6 weeks (20-25 g) from SLAC
laboratory animal Co., Ltd (Shanghai, China) were fed with a standard diet and water *ad libitum*. In order to obtain peritoneal exudates, 10 mL of sterile phosphate buffer saline (PBS, pH =7.4, 4 °C) was intraperitoneally injected into mice in this experiment. Mice were soaked in 95% ethanol for 2 min after being sacrificed by cervical dislocation. 0,83% ammonium chloride was added to cleave the red blood cells in peritoneal exudates. Cell suspension was centrifuged at 1600 × *g* for 5 min at room temperature. Macrophages suspension in DMEM medium were seeded in the upper chamber of transwell (6 mm diameter, 5 μm pore size), while HBZY-1 cells were seeded in the basolateral side and co-cultured for 48 h. Then cotton was used to wipe gently non-passed macrophages in apical side. Sequentially, 10% crystal violet was used to stain the macrophages on microporous membranes for 10 min. Additionally, the membranes were photographed under microscope connecting to the imaging system. The crystallization was dissolved by adding 30% acetic acid of 100 μL. The solutions were transferred into 96-well plates for optical density (OD) value detection at 580 nm.

**4. Western blot analysis**

Western blot analysis was performed to further evaluate the effect of Pae and Oxypae on IL-6 and MCP-1 protein expressions as described previously [20]. Namely, cells were lysed with lysis buffer and centrifuged at 12,000 × *g* for 15 min; 70 micrograms of protein were separated by 10% SDS-PAGE gel. After being equilibrated in transfer buffer for 15 min, separated gel was transferred onto a PVDF membrane and then blocked overnight with 5% non-fat milk at 4 °C. Subsequently, the probe was incubated with the primary antibodies anti-IL-6, anti-MCP-1, and anti-β-actin (Santa Cruz Biotechnology) overnight at 4 °C. Additionally, membranes were washed thrice with TBS and incubated with HRP-bound
secondary antibody for 1 h at room temperature (1:5,000 dilution). An enhanced chemiluminescence kit was used for the visualization of the protein bands. The protein levels were quantified by Image pro plus software.
Fig. 1S Fluorescence staining for macrophages in microporous membranes. Red arrows show the migration of macrophages. 200 μg/mL BSA (a); 200 μg/mL AGEs (b); 10 AG (10 μM) + 200 μg/mL AGEs (c); Pae (10^{-4} M) + 200 μg/mL AGEs (d); Pae (10^{-5} M) + 200 μg/mL AGEs (e); Pae (10^{-6} M) + 200 μg/mL AGEs (f); Oxypae (10^{-4} M) + 200 μg/mL AGEs (g); Oxypae (10^{-5} M) + 200 μg/mL AGEs (h); Oxypae (10^{-6} M) + 200 μg/mL AGEs (i). Magnification: 200×.
**Fig. 2S** Western blot analysis for inflammation. 200 μg/mL BSA (a); 200 μg/mL AGEs (b); AG (10 μM) + 200 μg/mL AGEs (c); Pae (10^{-4} M) + 200 μg/mL AGEs (d); Pae (10^{-5} M) + 200 μg/mL AGEs (e); Pae (10^{-6} M) + 200 μg/mL AGEs (f); Oxypae (10^{-4} M) + 200 μg/mL AGEs (g); Oxypae (10^{-5} M) + 200 μg/mL AGEs (h); Oxypae (10^{-6} M) + 200 μg/mL AGEs (i).