¹Qianping Gao, ²Fengping Wang, ¹Shuyuan Guo, ¹Jiali Cheng, ²Yinghua Jin, ²Bo Li, ¹Ye Tian, ¹Ye Tian. ¹The First Affiliated Hospital of Harbin Medical University; ²The Second Affiliated Hospital of Harbin Medical University

Objectives Emodin has been used as an anti-inflammatory agent, and inflammation is a crucial feature of atherosclerosis. Here, we investigated the sonodynamic effect of emodin on macrophages, the pivotal inflammatory cells in atherosclerotic plaque.

Methods THP-1 derived macrophages were cultured with emodin for 2 h and then exposed to ultrasound for 15 min. Two-hours later, immunofluorescence staining was performed to determine the cytoskeletal protein polymerisation. Six-hours later, Hoechst-PI staining was applied to distinguish the normal, apoptotic and necrotic cells. At the same time, MTT assay was performed.

Results Two-hours after treatment for 15 min, control cells showed a regular cytoskeletal network, and nuclei showed uniform fluorescence. There were no obvious morphological changes of the cytoskeleton in cells treated with emodin alone. The fluorescence signal of cytoskeletal protein was slightly attenuated 2 h after ultrasound exposure in some cells. In the case of cells treated with emodin-SDT, α -actin, β -tubulin and vimentin filaments dispersed and the proteins aggregated. The cytoskeleton lost its original features. Six-hours after emodin-SDT, the viability of cells treated for 15 min decreased significantly. Cell viability decreased significantly to $54 \pm 5\%$ in cells treated with emodin-SDT. Cell viability decreased $72 \pm 9\%$ in cells treated with ultrasound alone. Cell viability decreased more significantly in cells treated with emodin-SDT for 15 min than ultrasound alone ($p < 0.01$). Treatment with emodin alone did not affect cell viability compared to the control ($p > 0.05$). At the same time, the cells showed typical apoptotic chromatin fragmentation. The controls and cells treated with emodin alone showed uniform blue fluorescence; apoptotic cells were seen as bright blue fluorescence spots, and necrotic nuclei were identified by the presence of staining with PI, which was evident as pink fluorescence. The percentages of apoptotic cells in the ultrasound group and the SDT group were higher than that of the control ($26 \pm 6\%$ vs $4 \pm 3\%$, $p < 0.01$; $32 \pm 6\%$ vs $4 \pm 3\%$, $p < 0.01$, respectively). The percentage of apoptotic cells in the SDT group was higher than that in the ultrasound group ($32 \pm 6\%$ vs $26 \pm 6\%$, $p < 0.05$). The percentages of necrotic cells in the ultrasound group and the SDT group were higher than that of the control ($5 \pm 2\%$ vs $2 \pm 1\%$, $p < 0.05$; $17 \pm 5\%$ vs $2 \pm 1\%$, $p < 0.01$, respectively). The percentage of necrotic cells in the SDT group was higher than that in the ultrasound group ($17 \pm 5\%$ vs $5 \pm 2\%$, $p < 0.01$). There was no discernible difference in the percentage of apoptotic and necrotic cells between the emodin treated group and the controls.

Conclusions Emodin induces the apoptosis and necrosis of macrophages under ultrasound exposure. The results imply emodin-SDT might be a potential treatment for atherosclerosis by reducing the infiltration of macrophages in atherosclerotic plaque.