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Objectives Senescence of endothelial cells has been proposed to play an important role in endothelial dysfunction and atherogenesis. In the present study we aimed to investigate whether Tongxinluo (TXL) capsule protects human umbilical vein endothelial cells (HUVECs) from H₂O₂-induced endothelial senescence.

Methods Isolation, culture and identification of HUVECs: With the informed consents of puerperants, the normal fetal umbilical cords were obtained through uterine-incision delivery in Third Affiliated Hospital, Sun Yat-sun University. HUVECs were isolated by Percoll density gradient centrifugation from fetal umbilical cords with digestion of collagenase type I perfusion, and then suspended in Medium 199 cultured in 0.05 volume fraction of CO₂ incubator at 37°C. After the HUVECs were identified by flow cytometry with the cell marker CD31, The second or third passage was used for study. The exponentially growing HUVECs were plated at a cell density of 1×10⁵/well in 6-well plate and cultured overnight at 37 °C. The next day, the medium was changed with M199 supplemented with 2% FBS for at least 8 h to starve the cells. Then the cells were exposed to various concentrations of H₂O₂ to induce premature senescence. After 1 h, the medium was replaced with normal medium. Different concentration of TXL (0.1, 0.5, 1.0, and 2.0 mg/ml) was added in the media 30 min before the induction of senescence by addition of H₂O₂. Finally the cells were harvested in indicated time for Western blot analysis and real-time polymerase chain reaction (PCR). The MDA level and SOD activity were determined using commercially available kits following the manufacturer's instructions. Intracellular ROS generation was monitored by flow cytometry using peroxide sensitive fluorescent probe 2',7'-dichlorofluorescein diacetate (H₂DCFDA, Invitrogen).

Results

- (1) Treatment with H₂O₂ caused significant increase in intracellular thiobarbituric acid reactive substances (TBARS) level ($p < 0.01$ vs untreated control), while pre-incubation with TXL (0.5, 1.0 mg/ml) markedly attenuated the increase ($p < 0.05$). Compared to control group, treatment with H₂O₂ decreased the activity of SOD to 50.4±6.9%. However, pre-incubation with TXL (0.1, 0.5, 1.0, and 2.0 mg/ml) significantly increased SOD activity compared with H₂O₂ alone treated group ($p < 0.01$).
- (2) Real-time PCR analysis showed that at the time point of 24 h, SOD1 mRNA in the 60mmol/l H₂O₂ treated group decreased by 1.99 fold, compared with untreated group ($p < 0.01$). However, SOD1 mRNA increased by 1.54, 1.77, 1.75 fold in 0.1, 0.5, 1.0mg/ml TXL pretreated group respectively, compared with H₂O₂ alone treated group ($p < 0.01$).
- (3) Western blotting results demonstrated that the level of SOD1 protein reduced in H₂O₂ treated group compared to untreated group after 24, 48 and 72 h incubation. However, TXL reversed the decreased expression of SOD1 protein in HUVECs treated with H₂O₂ in a dose-dependent manner. As the control, TXL alone did not significantly change the expression of SOD1 at either mRNA or protein level.
- (4) TXL alone did not change the ROS generation. However, compared to the nontreated control group, 60 mmol/l H₂O₂ significantly increased DCF fluorescence whereas pre-treatment with 1.0 mg/ml TXL markedly inhibited the production of ROS induced by H₂O₂.

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TONGXINLUO CAPSULE PROTECTS ENDOTHELIAL CELLS FROM HYDROGEN PEROXIDE-INDUCED CELL SENESCENCE BY MODULATING REDOX STATUS

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Conclusions our data demonstrate that ginseno side TXL modulates redox status such as upregulating SOD1 expression, scavenging ROS, and decreasing the peroxidation to prevent the cellular senescence in HUVECs.