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Abstract

**Inhibitory effect of Cryptotanshinone and Tanshinone I on
TNF-alpha-induced adhesion molecule expression in human
umbilical vein endothelial cells**

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Background: Atherosclerosis has been the number one killer for people's health. It's been proved that inflammation is the main mechanism of atherosclerosis, and the upregulation of adhesion molecule expression on vascular endothelium, such as ICAM-1, VCAM-1 and E-selectin, play an key role in the progression of inflammation.

The upregulated expression of cell adhesion molecules such as ICAM-1, VCAM-1 and E-selectin on the endothelial cells alters the adhesive property of the vasculature, and it is one of the key events that leads to infiltration of leukocytes across the blood vessels and hence causes inflammation. A critically regulated expression of cell adhesion molecules is therefore essential for maintaining normal homeostasis of the body.

Salvia miltiorrhiza is an effective Chinese herb on prevention and treatment of cardiovascular and cerebrovascular diseases with the fuction of promoting blood circulation and removing blood stasis. Since most of studies on *Salvia miltiorrhiza* have mainly focused on its hydrophilic compounds, the beneficial effect of the liposoluble constituents such as Cryptotanshinone and Tanshinone I which are

comparatively more difficult to be characterized due to its poor solubility and tiny quantity, from *Salvia miltiorrhiza* are essentially to be explored. The study is to investigate the effect of Cryptotanshinone and Tanshinone I on anti-vascular inflammation *in vitro* and the underlying mechanism of action. The finding of this study should provide rationales and clues for further development of *Salvia miltiorrhiza* on prevention and treatment of vascular inflammation associated diseases such as atherosclerosis.

Objective: To illustrate the mechanism of anti-vascular inflammatory effects of Cryptotanshinone and Tanshinone I isolated from root of *Salvia miltiorrhiza* by determining the effect of Cryptotanshinone and Tanshinone I on the expression of ICAM-1, VCAM-1 and E-selectin in the TNF- α -stimulated human umbilical vein endothelial cells (HUVEC).

Methods: Firstly, the cytotoxicity of Cryptotanshinone and Tanshinone I on HUVEC cells were evaluated by XTT cell proliferation assay. Then, TNF- α induced HUVEC inflammation model was evaluated the anti-inflammatory effect of the investigated compounds. The expression of ICAM-1, VCAM-1 and E-selectin were determined by cell-ELISA, immunofluorescent staining and western blot while mRNA expression of ICAM-1, VCAM-1 and E-selectin were determined by RT-PCR. Moreover, the nuclear translocation of NF- κ B p65 protein was determined by immunofluorescent staining. The data was expressed as mean $\pm$ S.E.M. of more than three independent experiments.

Results: Cryptotanshinone at a concentration of higher than 10 μ M showed cytotoxic effect on HUVEC cells. TNF- α significantly increased the expression of ICAM-1, VCAM-1 and E-selectin in HUVEC cells ;but

Cryptotanshinone and Tanshinone I alone, below non-cytotoxic concentration, could inhibit the inducible mRNA and protein expression of ICAM-1, VCAM-1 and E-selectin on the TNF- α activated HUVEC cells. We also found that the nuclear translocation of NF- κ B p65 protein was inhibited by the pre-treatment with Cryptotanshinone especially at the concentration of 1 μ M and 3 μ M., and Tanshinone I also inhibit the nuclear translocation of NF- κ B p65 protein at the concentration of 5 μ M and 3 μ M.

Conclusions: This investigation suggested that Cryptotanshinone and Tanshinone I had strong anti-inflammatory effect by inhibiting the expression of ICAM-1, VCAM-1, E-selectin and the nuclear translocation of NF- κ B p65 protein, in TNF- α activated HUVEC cells and may play an important role in the therapeutic effect of *Salvia miltiorrhiza* on atherosclerosis and inflammatory responses.

Keywords: Cryptotanshinone ; Tanshinone I; human umbilical vein endothelial cells; adhesion molecule ; inflammation