Inhibitory effect of three isoflavonoids from *Radix Puerariae* on TNF-alpha-induced adhesion molecule expression in human umbilical vein endothelial cells (HUVEC)

Abstract：Atherosclerosis (AS) is a chronic and complex vascular inflammatory disease characterized by lipid deposition and even lipid plaque formation in the vascular intima. Both the etiology and the pathology of the disease remain unclear and several mechanisms had been proposed such as the lipid infiltrative theory, the thrombosis hypothesis, the platelet aggregation theory, and the clone hypothesis as the cause of this disease. Now, in general, most researchers believed that the pathogenesis of AS is the complicated results of the interaction among the vascular cells, extracellular matrix, blood constituents (especially monocytes, platelets and low density lipoprotein (LDL)), local hemodynamic environment and heredity. Therefore, there is no single etiological factor for AS. At present, the general view of the pathogenesis of AS is that AS is the complicated interaction among injury, inflammation and immune dysfunction.

Due to the complicated pathogenesis of AS which includes many links and drug targets, the development of effective drugs which act on these multiple specific targets for AS prevention and therapy is slow unavailable. Preclinical and clinical studies revealed that traditional Chinese medicine were comprised of multiple chemical components and have mutli- therapeutic targets and showed good therapeutic effect in AS prevention and therapy. However, the action mechanisms of most Chinese medicine were still unclear yet. More reports suggested that Gegen (*Radix Puerariae).*, a Chinese medicine showed good anti-atherosclerosis effects but the underlying mechanism of action need to be elucidated. In this manuscript, HUVECs were used to explore the possible anti-atherosclerosis mechanisms of action of isoflavonoids including daidzein, daidzin and puerarin from Gegen. The effects of daidzein, daidzin

and puerarin on TNF-α induced ICAM-1 and VCAM-1 expression were tested by ELISA, immunofluorescence staining and Western blotting. Their inhibitory effects on cell-cell adhesion between endothelial cells and monocytes were also determined. Results showed that no significant cytotoxicity was observed for treatment with 1-100μM puerarin. At 1-30μM neither daidzein nor daidzin showed cytotoxicity to endothelial cells while both of them showed obvious cytotoxicity at 100μM. Cell-ELISA results revealed that all of these three isoflavonoids compounds from Gegen could significantly inhibited TNF-α induced ICAM-1 expression in, a dose-dependent manner. Immunofluorescence staining and Western blotting showed similar results as observed in cell-ELISA. In summary, all these results suggested that Gegen (*Radix Puerariae)* may reduce atherosclerosis through inhibiting endothelial ICAM-1 and VCAM-1 expression .

Keywords: Puerarin, Daidzin, Daidzein, HUVEC, Anti-atherosclerosis.

摘要

動脈粥樣硬化(Atherosclerosis, AS)是以富含脂肪的斑塊在大動脈壁聚積爲特徵的系統病變,是心腦血管病的主要病理基礎。動脈粥樣硬化形成原因相當複雜,現代醫學關於AS的病因較一致的看法是由損傷、炎症、免疫功能障礙三者相結合作用的結果[1]。其主要臨床表現是心肌梗塞、中風和外周血管疾病,常伴有高血壓、高膽固醇血症或糖尿病等。動脈粥樣硬化導致的疾病是發達國家第一位的死亡原因[2]。在我國,隨著經濟社會的發展和人口的老齡化,心腦血管病發病率與死亡率近年也顯著增加。

中藥含有的諸多成分,具有多環節、多靶點作用的性質,對機制複雜的動脈粥樣硬化有更好更全面的防治作用。越來越多的報導說明中藥葛根具有抗動脈粥樣硬化的藥理作用,但在葛根怎樣抗動脈粥樣硬化作用上還沒有清晰的闡釋。本