EFFECTS OF ACTIVE PRINCIPLES FROM S. MILTIORRHIZA ON HUMAN ENDOTHELIAL CELLS AND PLATELETS

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Angiogenesis is a multi-factorial process involving endothelial cells, pericytes, macrophages, angiogenic factors and inhibitors. It plays a fundamental role in many physiological and pathological processes such as wound healing and atherosclerosis. Haemostasis is the arrest of blood loss from damaged blood vessels involving platelet adhesion, activation and blood coagulation. Recent studies suggest these two processes are closely related, because some proteins derived from platelets or the coagulation cascade are involved in controlling angiogenesis (Carmeliet, 2003; Shi \textit{et al.}, 2005).

Despite limited knowledge on its mechanisms of action, \textit{Salvia miltiorrhiza} has been widely used in Asia for several hundred years for the treatment of angiogenesis- and haemostasis-related diseases, such as atherosclerosis, thromboembolism and angina. We speculate that the active principles [e.g., cryptotanshinone (CT) and tanshinone IIA (T\textsubscript{2}A)] derived from this medicinal plant would exert distinct pharmacological actions on endothelial and platelet biology. A clear understanding of their mechanisms of action could lead to novel compounds for the treatment of angiogenic diseases.

Using the MTS tetrazolium cell proliferation assay (Promega), we showed CT and T\textsubscript{2}A to inhibit ($n = 3$, $P \leq 0.05$) the proliferation of human umbilical vein endothelial cells (HUVECs) in a concentration-dependent manner (1-100\textmu M), with IC\textsubscript{50} of 1.65 \textmu M and 1.48 \textmu M, respectively. In a modified Matrigel assay, both compounds inhibited HUVEC tube formation at 5\textmu M ($n = 3$). To elucidate these anti-angiogenic activities of CT and T\textsubscript{2}A, apoptosis was evaluated by caspase 3/7 assay. The results showed a 110\pm8% increase in caspase 3/7 activity in HUVEC after 24h treatment with 10\textmu M T\textsubscript{2}A, while \textmu M CT only increased caspase activity by 22\pm10%. Thus, T\textsubscript{2}A induced HUVEC apoptosis, but CT did not. Flow cytometry using propidium iodide/annexin V staining revealed that 84% of HUVECs treated with 10\textmu M CT accumulated in G\textsubscript{1}/G\textsubscript{0} phase compared with 72% in the control. Similar results were obtained in two independent experiments.

In human platelets, CT and T\textsubscript{2}A did not show any intrinsic activity to induce aggregation. Pretreatment of platelets with CT and T\textsubscript{2}A (0.5-50\textmu M) for 15 min effectively blocked the secondary wave of the ADP-induced platelet aggregation ($n = 3$, $P \leq 0.05$), with IC\textsubscript{50} of 150 nM and 90 nM, respectively. Interestingly, CT and T\textsubscript{2}A did not affect noradrenaline- or collagen-induced aggregation. ($n = 3$, $P \geq 0.05$)

Taken together, we demonstrate that CT and T\textsubscript{2}A exert distinct pharmacological effects on human endothelial cells and platelets, and offer some insights on the cellular mechanisms of action of these compounds. Further work aiming to elucidate their molecular mechanisms and to evaluate their in vivo pharmacology would provide a novel platform of drug discovery for the prevention and treatment of cardiovascular and cerebrovascular diseases.