MASTER'S THESIS

A comparative study of two anti-angiogenic compounds: sinomenine and norcantharidin

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A Comparative Study of Two Anti-angiogenic Compounds: Sinomenine and Norcantharidin

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Abstract

Angiogenesis is the development of new blood vessels from existing vasculature. Excessive angiogenesis is one of the pathological symptoms of rheumatoid arthritis (RA) and tumour development. Sinomenine has been used for treatment of rheumatoid arthritis while norcantharidin possesses anti-tumour effect. In this study, the anti-angiogenic effect of sinomenine and norcantharidin (NCTD) were investigated.

Sinomenium acutum is a Chinese herb that has been utilized to treat rheumatoid arthritis (RA) and related diseases in China for many years. Sinomenine (7,8-didehydro-4-hydroxy-3,7-dimethoxy-17-methylmorphinane-6-one) is an alkaloid which has a chemical structure very similar to morphine. It has several pharmacological activities, such as anti-inflammatory, immunosuppressive and anti-arrhythmia effect.

In first part of my study, sinomenine was found to suppress angiogenesis in two different *in vivo* assays, Matrigel plug assay (150 μM) and micro-vessels formation in rat aorta ring sprout assay (125 μM). The anti-angiogenic effect of sinomenine was further investigated *in vitro* with human umbilical vein endothelial cells (HUVEC). Sinomenine inhibited the proliferation of HUVEC and arrested the cell cycle progression in G1 phase (IC_{50}=1 mM). In addition, it disrupted capillary-like structure formation on Matrigel (1 mM) and inhibited basic fibroblast growth factor (bFGF)-induced HUVEC chemotaxis (300 μM). It is suggested that sinomenine inhibits bFGF-induced angiogenesis both *in vitro* and *in vivo*. Since bFGF is a chronic angiogenic factor that promotes angiogenesis in RA patients, the

anti-angiogenic effect of sinomenine may improve RA treatment.

Endothelial cells play a role in leukocytes trafficking in the development of inflammatory disease. It adheres and recruits circulating leukocytes from the blood stream. In this study, the anti-inflammatory effect of sinomenine was studied with trans-endothelial migration assay. Pre-incubation of differentiated HL60 cells with sinomenine (1 mM) significantly reduced the transmigration of HL60 across IL-1β activated HUVEC.

Furthermore, angiogenesis is essential for blood supply, waste removal of solid tumour and tumour cells metastasis. In the absence of neovascularization, tumour lays dormant and unable to grow beyond 2 to 3 mm^3 in size. Recent research indicated that if anti-tumour drugs were applied continuously at the low dosage level, it would affect the tumour vasculature. Norcantharidin (NCTD), a demethylated form of cantharidin with anti-tumour activities, has been used in the treatment of patients with hepatoma in China. However, the mechanistic actions of NCTD on other cancer cells are still unknown. In second part of my study, both anti-angiogenic and anti-tumour effects of NCTD were studied using nasopharyngeal carcinoma (NPC) models.

The anti-angiogenic effect of NCTD was investigated *in vitro* using human umbilical vein endothelial cells (HUVEC). NCTD inhibited HUVEC proliferation (IC_{50}= 65 μM) and disrupted tube formation on Matrigel (10 μM). It suppressed bFGF - induced angiogenesis in *in vivo* Matrigel plug assay (20 μM).

The anti-tumour activity of NCTD was evaluated with both NPC/HONE-1 and

NPC/CNE-2 cells. It is cytotoxic towards nasopharyngeal carcinoma cells NPC/HONE-1 (LC_{50}= 250 μM) and NPC/CNE-2 (LC_{50}= 100 μM). Treatment of NPC/CNE-2 cells with NCTD blocked the cell cycle progression into G2/M phases and induced DNA fragmentation within 24 hours, the apoptotic process was characterized with the activation of caspases-3,-8,-9 and-12. Activation of caspase-8 suggested apoptosis was mediated by TNF and Fas receptors. However, TNF and Fas ligand neutralising antibodies were unable to reduce the NCTD cytotoxicity. The effect of NCTD on mitochondria damage was investigated. The results indicated that NCTD induced mitochondria stress with depolarization of mitochondria membrane potential ($\Delta \Psi_m$), followed by activation of caspase-9 and caspase-3 activation and DNA fragmentation. Finally, NCTD also cause damage of endoplasmic reticulum resulting in activation of caspase-12 apoptosis. Taken together, it is suggested that NCTD suppressed tumour development through the induction of tumour cells apoptosis and blockage of angiogenesis.

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