

EVALUATION OF NEW ANDROGRAPHOLIDE DERIVATIVES FOR INHIBITORY ACTIVITIES AGAINST TUMOUR VASCULATURE AND METASTASES

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ABSTRACT

Andrographolides were isolated from the herb *Andrographis paniculata*. Using selected natural compounds as starting materials, a library of semisynthetic analogues was prepared. The compounds were evaluated for their inhibitory effects against tumour angiogenesis and metastases using *in vitro* and *in vivo* models. Inhibition of angiogenesis is currently perceived as one of the most promising strategies in the treatment of cancer. A natural compound termed CRDD-2 and its derivatives were investigated for their antiangiogenic effects *in vitro* and *in vivo*. The compounds showed significant differential cytotoxicity in human umbilical endothelial vein cells (HUVEC) and HT29 colon cancer cells. In addition, at highest non-cytotoxic concentrations, the test agents significantly inhibited HUVEC proliferation and capillary tube formation triggered by vascular endothelial growth factor (VEGF) *in vitro*. To determine the mechanism of action CRDD-2 in inhibiting the HUVEC proliferation, the down-stream mitogen-activated protein kinase signal transduction pathways that are associated with VEGF-triggered HUVEC proliferation were investigated. We demonstrated that CRDD-2 inhibited VEGF-induced extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation in a dose-dependant manner as compared with VEGF control. In an animal study, no overt tissue toxicity or significant weight loss greater than 15% was observed in mice treated with daily treatment of CRDD-2 at 400 mg/kg for 14 days. Pharmacokinetic parameters showed CRDD-2 was quickly absorbed into circulation after intraperitoneal (i.p) administration of single-doses CRDD-2 at 300 mg/kg and 600 mg/kg with T_{max} of 5 minute and plasma concentration range of 20 – 350 μ M corresponding to *in vitro* antiangiogenic and possibly cytotoxic activities. Daily treatment of 300 mg/kg CRDD-2 for 14 days displayed a weak *in vivo* antitumor activity against HT29 tumour xenografts and escalation of dose to 400 mg/kg slowed 2-fold tumour growth approximately by 4.2 days. *In vivo* tumour angiogenesis was evaluated by immunohistochemical staining of endothelial cell marker CD31. The results indicated that treatment with 400 mg/kg of CRDD-2 reduced the CD31-positive area in viable regions of HT29 tumour tissues by 26% as compared with the vehicle control group. Taken together, these results

demonstrate CRDD-2 and its semisynthetic derivatives possess antiangiogenic activity against endothelial cells which could be exploited for inhibition of tumour angiogenesis leading to tumour growth inhibition.

The anti-invasive and anti-metastatic properties of CRDD-2 and its semisynthetic derivatives were investigated using various bioassays. The anti-invasive activity of the compounds was examined using *in vitro* transwell assay. Treatments with non-cytotoxic concentrations of CRDD-2 derivatives, namely SRS28 and SRS49 were able to inhibit invasion of the highly metastatic mouse melanoma B16F10 cells. These results suggest that CRDD-2 derivatives possess anti-invasive property. Matrix metalloproteinases (MMPs) are zinc-dependent proteolytic enzymes, play a critical role in tumour invasion and metastasis by degrading extracellular matrix. High expression of MMPs is correlated with tumour invasiveness and unfavourable cancer prognosis. Zymographic analysis showed that SRS28 and SRS49 significantly suppressed MMP-2 activities in a concentration-dependent manner. This is highly suggestive that the compounds' antiinvasive effect against B16F10 mouse melanoma cells could be related to their ability to suppress the activity of MMP-2. Agents with antiinvasive effect have strong potential as antimetastatic agents. As SRS28 was the most promising agent, it was selected for evaluation of *in vivo* antimetastatic activity in a C57BL/6 mice inoculated with B16F-10 melanoma cells. Preliminary results indicated SRS28 had the ability to reduce tumour incidence in the lungs of the animals. In conclusion, SRS28 and SRS49 serve as potential lead compounds with inhibitory activity against tumour invasion and metastasis.