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Licorice isoliquiritigenin dampens angiogenic activity via inhibition of MAPK-responsive signaling pathways leading to induction of matrix metalloproteinases

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Abstract

The aberrant expression of matrix metalloproteinases (MMPs) has been implicated in matrix degradation leading to angiogenesis. This study examined the inhibitory effects of isoliquiritigenin (ISL) on phorbol myristate acetate (PMA)-induced MMP production and its tissue inhibitor of MMP (TIMP) in endothelial cells. No induction of either necrotic or apoptotic cell death was observed in response to a treatment with ISL at ≤25 μM. ISL dose-dependently suppressed PMA-induced expression and activity of MMP-2 and membrane type 1-MMP at ≥1 μM while diminishing the elevated MMP-2 transcript level. In addition, ISL inhibited PMA-triggered migration and tube formation in a dose-dependent manner. ISL further increased the TIMP production up-regulated by PMA with a biphasic effect on TIMP-2 expression. This study further attempted to investigate whether a c-Jun N-terminal kinase (JNK)- or p38 mitogen-activated protein kinase (MAPK)-responsive mechanism was responsible for the MMP production and whether ISL disturbed these signaling pathways. PMA stimulated signaling of JNK and p38 MAPK, which was dampened by ≥10 μM ISL. These results demonstrate that ISL blocked JNK- or p38 MAPK-responsive pathways leading to direct MMP activation of PMA-exposed endothelial cells. Therefore, the ISL inhibition of MMP may boost a therapeutic efficacy during angiogenesis.

Keywords: Angiogenesis; Isoliquiritigenin; MMP-2; MT MMP-1; PMA; Tube formation

1. Introduction

Matrix metalloproteinases (MMPs) play an important role in tumor invasion, angiogenesis and inflammatory tissue destruction [1,2]. Increased expression of MMP was observed in benign tissue hyperplasia and in atherosclerotic lesions [2,3]. These enzymes may contribute to a cell-invasion-favoring matrix modification and, thus, to an invasive aggressiveness of tumor cells [4,5]. Invasive cancer cells utilize MMP to degrade the extracellular matrix (ECM) and basement membrane during metastasis, and MMP-2 has been implicated in the development and dissemination of malignancies [6]. The interaction between malignant cells and peritumoral benign tissues including the vascular endothelium may serve as an important mechanism in the regulation of tumor invasion and metastasis [6]. Their proteolytic activity is regulated by inhibitors or activators such as tissue inhibitors of MMP (TIMPs), membrane-type MMP (MT MMP) and urokinase-type plasminogen activator (uPA) [1,7,8].

Licorice is a flavorful herb that has been used in food and medicinal remedies for thousands of years. Its root has been used in both Eastern and Western medicine to treat a variety of illnesses ranging from the common cold to liver diseases [9]. Licorice root contains flavonoids such as flavans and chalcones [10–12]. Glabridin, an isoflavan from licorice root, improves survival of mice in an experimental model of septic shock through inhibiting expression of inducible nitric oxide

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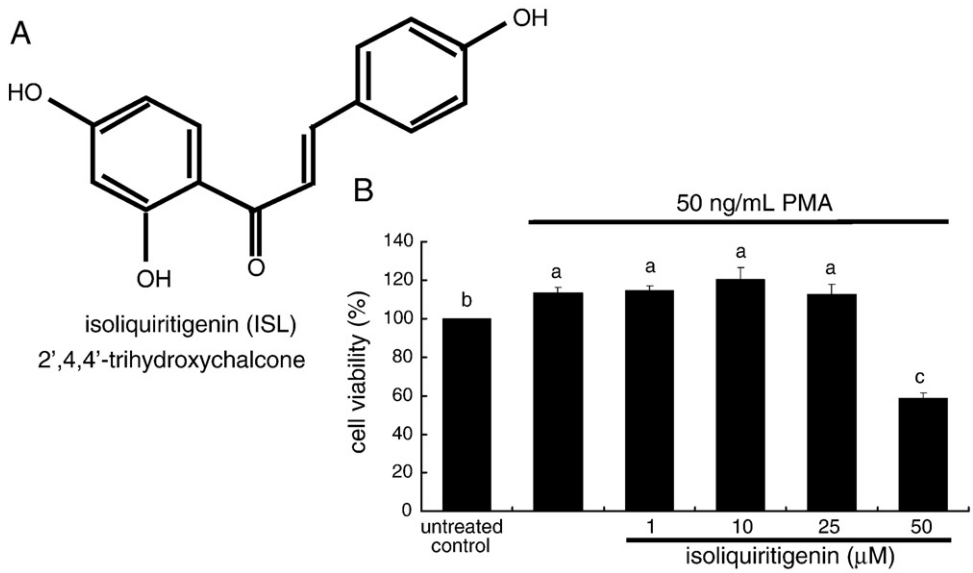

Fig. 1. Chemical structures (A) and cytotoxicity (B) of ISL. After HUVECs were cultured for 24 h in the presence of 50 ng/ml PMA and 1–50 μM ISL, MTT assay was performed. The bar graph data represent means±S.E.M. from five independent experiments with multiple estimations. Values are expressed as percentage of cell survival relative to untreated control cells (cell viability=100%). Values not sharing a letter are different at $P<.05$.

synthase [12]. There is growing interest on the beneficial health effects of isoliquiritigenin (ISL) in licorice root with a chalcone structure, 2′,4,4′-trihydroxychalcone (Fig. 1A), due to its antioxidative, antitumor and anti-inflammatory activities [13,14]. ISL is regarded as a promising potential cancer chemopreventive agent [13,15]. Previous studies have shown that ISL induces apoptosis in colon, gastric and prostate cancer cells [15–17]. However, the antitumor mechanisms of ISL have not been well defined. There is little literature regarding MMP's involvement in matrix modification of ISL as a chemopreventive mechanism.

Licorice-derived flavonoids at micromolar concentrations inhibited tube formation from vascular endothelial cells and granuloma angiogenesis; their potencies for anti-tube formation were in the following order: ISL>isoliquiritin>liquiritigenin≫isoliquiritin apioside [19]. Based on the literature evidence that licorice may inhibit angiogenesis and vascular tumor growth [18,19], the present study determined whether ISL prevents migration and tube formation via inhibition of MMP-2 induction in phorbol 12-myristate 13-acetate (PMA)-exposed human umbilical vein endothelial cells (HUVECs). This study further attempted to elucidate action mechanisms responsible for the MMP activation and for the ISL inhibition of its induction.

2. Materials and methods

2.1. Materials

M199 medium chemicals, ISL and 3-(4,5-dimetylthiazolyl)-diphenyl tetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO), as were all other reagents, unless specifically stated elsewhere. Collagenase was purchased from Worthington Biochemicals (Lakewood, NJ). Fetal bovine serum (FBS), penicillin–streptomycin, trypsin–EDTA, human epidermal growth factor and hydrocortisone were purchased from Cambrex (East Rutherford, NJ). Proteins MMP-2 and MT-1 MMP and antibodies against human MMP-2, MT-1 MMP and TIMP-2 were purchased from R&D Systems (Minneapolis, MN). In addition, antibodies against human phospho-c-Jun N-terminal kinase (JNK), human phospho-p38 mitogen-activated protein kinase (MAPK), human phospho-c-Jun and human phospho-p53 were obtained from Cell Signaling Technology (Beverly, MA). Human β-actin antibody was purchased from Sigma Chemicals. Horseradish-peroxidase-conjugated goat anti-rabbit IgG, donkey anti-goat IgG and goat anti-mouse IgG were obtained from Jackson ImmunoResearch Laboratories (West Grove, PA). PMA was purchased from Sigma-Aldrich. Reverse transcriptase, *Taq* DNA polymerase and dNTP were purchased from Promega (Madison, WI). ISL was solubilized in dimethyl sulfoxide (DMSO) for culturing with cells; the final culture concentration of DMSO was ≤0.05%.

2.2. Preparation and culture of human endothelial cells

HUVECs were isolated from umbilical cords using collagenase as described elsewhere [14,20]. Cultures were maintained at 37°C humidified atmospheres of 5% CO_2 in air. Cells were cultured in 25 mM HEPES-buffered M199 containing 10% FBS, 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin supplemented with 0.75 mg/ml human epidermal growth factor and 0.075 mg/ml hydrocortisone.

HUVECs were plated at 90–95% confluence in all experiments. In experiments for the PMA-induced secretion