Isoliquiritigenin inhibited cell proliferation and triggered apoptosis in human endometrial cancer cell line

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Objective: To determine the anti-tumor effect of isoliquiritigenin (ISL) on endometrial cancer cell and to evaluate its effect on apoptosis in Hec1A endometrial cancer cell lines.

Methods: Human endometrial cancer cell lines (Hec1A) and Ishikawa, and normal endometrial cell line (T-HESCS) were cultured in vitro. The viabilities of three cell lines on ISL were measured. Cell cycle distribution and induction of apoptosis were measured in Hec1A cells after ISL treatment.

Results: ISL significantly reduced cell viabilities of endometrial cancer cell lines but not normal cell line in a dose-dependent manner. Cell cycle analysis indicated that ISL treatment increased the proportion of cells in the sub-G0/G1 phase. DNA fragmentation and fluorometric TUNEL assays also revealed apoptotic cell death after ISL incubation. ISL treatment markedly up-regulated the expression of cyclin-dependent kinase inhibitor, p21 Cip1/Waf1 in a p53 independent manner and down regulated the expressions of cyclins and CDKs, with concomitant increase in FAS and cleavage of caspase 7, caspase 8, and caspase 9. In addition, elevation of caspase 3 activity also observed in a dose and time dependent manner.

Conclusion: ISL inhibited cell proliferation and triggered apoptosis in human endometrial cancer cell line Hec1A. Hence, ISL can be used as a potentially potent clinical chemotherapeutic agent for treating endometrial cancer.

Key words: Endometrial cancer cell line; Hec1A; Isoliquiritigenin; Apoptosis

Introduction

Endometrial carcinomas are divided into two pathogenetic groups based on their molecular and clinical characteristics.¹ Type I, endometrial cancer, represents the majority of cases and occurs in most pre- and peri-menopausal women exposed to excess levels of estrogen.² Type II, endometrial cancers, arises from atrophic endometrium in older women, and is not associated estrogen exposure.² In recent years endometrial cancer has become the most common gynecologic malignancy in many developed countries.³ Although the primary treatment in endometrial cancer is surgical dissection, adjuvant chemotherapy has also been accepted as an effective therapeutic strategy in high risk or advanced endometrial cancer.⁴

In recent years, herbal therapies are commonly used in Western countries, but little is known about their effectiveness, mode of action and side effects. A lot of reports have shown the anti-carcinogenic property of plant polyphe-
Flavonoids, a plant pigment, are a group of naturally occurring polyphenolic substances found in fruits and vegetables and reported to have chemotherapeutic property. Over 4,000 flavonoids have been discovered and categorized, such as flavon, flavonol, flavanone, flavanol, isoflavone, chalcone, anthocyanin, and catechin according to its structure. Isoliquiritigenin (ISL) is a chalcone flavonoid, richly present in licorice (a legume) and shallot (a liliaceae). Our previous study demonstrated that Spatholobus subrectus Dunn possess inhibitory property in human uterine leiomyoma cells (unpublished data). ISL is one of the active components in Spatholobus subrectus Dunn and involved in biochemical pathways such as antioxidative and superoxide scavenging, antiplatelet aggregation, and anti-estrogenic activity. ISL is also reported to have anti-carcinogenic potencies such as anti-angiogenic and apoptosis inducing activity.

In the present study, we examined the anti-tumor effect of ISL on an endometrial cancer cell lines using cell cycle distribution and apoptosis analysis. This may offers a new promising therapeutic approach in overcoming endometrial cancer.

Materials and Methods

1. Cell culture

Human endometrial cancer cell line (Hec1A) and normal endometrial cell line (T-HESCS) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Ishikawa, human endometrial cancer cell line, was purchased from Sigma (St. Louis, MO, USA). Cells were cultured in Dulbecco's Modified Eagle Medium, DMEM/F-12, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin antibiotic mixture and grown to confluence. All the cell culture media and supplements were obtained from GibcoBRL (Grand Island, NY, USA).

2. Cell viability assay

To measure viability of ISL treated Hec1A cell, Tritiated Thymidine Incorporation (MTS) colorimetric assay (Promega, Madison, WI, USA) was performed. Hec1A cells seeded in a 96 well plate (2×10^4 cells/well) were treated with ISL (Sigma) which dissolved in dimethyl sulfoxide (DMSO). For control treatment (0 µM ISL) in overall experiment, 0.1% DMSO was used. After 48 hours incubation, MTS reagent was added and incubated for 4 hours at 37°C. Optical density was measured at 495 nm. Cell viability values were calculated relative to the control cells (100%) and expressed as means±standard error of three independent experiments.

3. Determination of cell cycle distribution

To determine cell distribution of the ISL treated Hec1A cells, FACS analysis was performed. After 48 hours of 50 µM ISL treatment, cells were harvested, washed with cold phosphate buffered solution (PBS), and were fixed in ice-cold 70% ethanol at 4°C for 24 hours. Then cells were treated with 0.1% RNaseA for 30 min at 37°C and were stained with propidium iodide and incubated for 30 min at room temperature. DNA fluorescence was measured by flow cytometer (FACSCalibur®, Becton Dickinson, Franklin Lakes, NJ, USA). The percentage of cells in each cell cycle phase was determined using the ModFit LT® software (Becton-Dickinson) based on the DNA histogram.

4. DNA fragmentation assay

Apoptotic cell death by ISL was determined using the Cellular DNA fragmentation ELISA kit (Roche Diagnostics, Penzberg, Germany), which determines the amount of cytoplasmic histone-associated DNA fragments. Cells (2×10^4/well) seeded on a 96 well plate were incubated with ISL for 48 hours then lyzed. The total lysates containing BrdU-labeled DNA fragments were transferred to anti-DNA antibody-coated microplates. Immobilized BrdU-labeled DNA fragments were measured at 370 nm after binding with anti-BrdU antibodies. Values were expressed as the mean of triplicate measurements.

5. Fluorometric TUNEL assay

DNA strand breaks due to apoptosis were also determined by DeadEnd Fluorometric TUNEL System assay kit (Promega). Hec1A cells were treated with 50 µM ISL for 48 hours, then fixed with formaldehyde and washed with PBS. Terminal Deoxynucleotidyl transferase was added to cells to incorporate fluorescein-12-dUTP at 3'-OH into DNA ends. Fluorescence-positive cells were analyzed by flow cytometer (FACSCalibur®).

6. Western blot analysis

Cell extracts were prepared in lysis buffer (10 mM Tris [pH 7.4], 5 mM EDTA, 130 mM NaCl, 1% Triton X-100, serine protease inhibitor phenylmethylsulphonyl fluoride [10 µg/mL], leupeptin [10 µg/mL], apro tinin [10 µg/mL], 5...