Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 17 (2006) 689–696
Journal of Nutritional Biochemistry

Isoliquiritigenin induces apoptosis by depolarizing mitochondrial membranes in prostate cancer cells☆

Jae In Jung[a], Soon Sung Lim[a,b], Hyun Ju Choi[a], Han Jin Cho[b], Hyun-Kyung Shin[a,b], Eun Ji Kim[b], Won-Yoon Chung[c], Kwang-Kyun Park[c], Jung Han Yoon Park[a,b,*]

[a]*Department of Food Science and Nutrition, Hallym University, Chuncheon 200-702, South Korea*
[b]*Silver Biotechnology Research Center, Hallym University, Chuncheon 200-702, South Korea*
[c]*Department of Oral Biology, College of Dentistry, Yonsei University, Seoul 120-752, South Korea*

Received 13 October 2005; received in revised form 8 November 2005; accepted 9 November 2005

Abstract

Isoliquiritigenin (ISL), a simple chalcone derivative, 4,2′,4′-trihydroxychalcone, found in licorice, shallot and bean sprouts, has been reported to have chemoprotective effects. To examine the effects of ISL on the growth of prostate cancer cells, we cultured MAT-LyLu (MLL) rat and DU145 human prostate cancer cells with various concentrations (0–20 μmol/L) of ISL. Treatment of the cells with increasing concentrations of ISL led to dose-dependent decreases in the viable cell numbers in both DU145 and MLL cells ($P<.05$). Hoechst 33258 dye staining of condensed nuclei and annexin V binding to surface phosphatidylserine revealed increased numbers of apoptotic cells after ISL treatment. Western blot analysis revealed that ISL increased the levels of membrane-bound Fas ligand (FasL), Fas, cleaved casapse-8, truncated Bid (tBid), Bax and Bad in DU145 cells ($P<.05$). Isoliquiritigenin increased the percentage of cells with depolarized mitochondrial membranes, in a concentration-dependent manner ($P<.05$). Isoliquiritigenin induced the release of cytochrome *c* and Smac/Diablo from the mitochondria into the cytoplasm ($P<.05$). Isoliquiritigenin dose-dependently increased the levels of cleaved caspsase-9, caspase-7, caspase-3 and poly(ADP-ribose) polymerase ($P<.05$). The present results indicate that ISL inhibits prostate cancer cell growth by the induction of apoptosis, which is mediated through mitochondrial events, which are associated with an evident disruption of the mitochondrial membrane potential, and the release of cytochrome *c* and Smac/Diablo, and the activation of caspase-9.

Keywords: Bcl-2; Caspase; Apoptosis; Cytochrome *c*; Prostate cancer cells

1. Introduction

Prostate cancer is the second leading cause of cancer-related deaths in the United States [1], and the incidence is also rapidly increasing in Asian countries. Androgen ablation has been the most frequently used treatment for disseminated prostate cancer, and most patients with a metastatic disease encounter an initial regression in response to this treatment. However, the disease eventually relapses in most patients and progressed to an androgen-independent state, which no longer responds to conventional therapy [2]. In the United States, prostate cancer is mainly found in men aged over 55 years, with the average age of patients at the time of diagnosis of 70 years. For this reason, it is necessary to increase efforts to gain a better understanding of and develop novel treatment and chemopreventive approaches for this disease.

Studies to identify agents with potentially preventive and therapeutic roles in cancer are increasing rapidly. Flavonoids, polyphenolic compounds ubiquitously present in plants, have drawn considerable interest due to their apparent ability to act as highly effective chemopreventive and chemotherapeutic agents [3]. Many of these compounds seem to act on multiple target signaling pathways in cancer cells [4].

Abbreviations: ISL, isoliquiritigenin; MLL, MAT-LyLu; PARP, poly-(ADP-ribose) polymerase.

☆ Supported by the Basic Research Program of the Korea Science and Engineering Foundation (R01-2004-000-10177-0), research grants from the Korea Science and Engineering Foundation (KOSEF) for Biofoods Research Program, Ministry of Science and Technology, and a research grant from Hallym University, Korea.

* Corresponding author. Department of Food Science and Nutrition, Hallym University, Chunchon 200-702, South Korea. Tel.: +82 33 248 2134; fax: +82 33 256 0199.
E-mail address: jyoon@hallym.ac.kr (J.H.Y. Park).

0955-2863/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2005.11.006

The ability of tumor cell populations to increase in number is determined not only by the rate of cell proliferation, but also by the rate of cell death. Because deregulated inhibition of apoptosis lies at the heart of all tumor development, it presents an obvious target for preventive and therapeutic intervention in all cancers [5]. Apoptosis is controlled by two major pathways, including the mitochondrial [6] and membrane death receptor pathways [7]. The great majority of cell death signals engage the mitochondrial pathway, where death signals lead to alterations in the mitochondrial membrane permeability, with the subsequent release of pro-apoptotic factors, such as cytochrome *c* and Smac/Diablo [6]. Cytosolic cytochrome *c* recruits and activates caspase-9 [8,9] by forming the macromolecular complex, apoptosome, which then processes and activates other caspases to induce apoptosis [10]. The Smac/Diablo released from the mitochondria during apoptosis functions to promote caspase activation by competing with caspases for binding of the inhibitor of apoptosis protein (IAP) family, thereby relieving the inhibitory effects of IAPs on caspases [11,12].

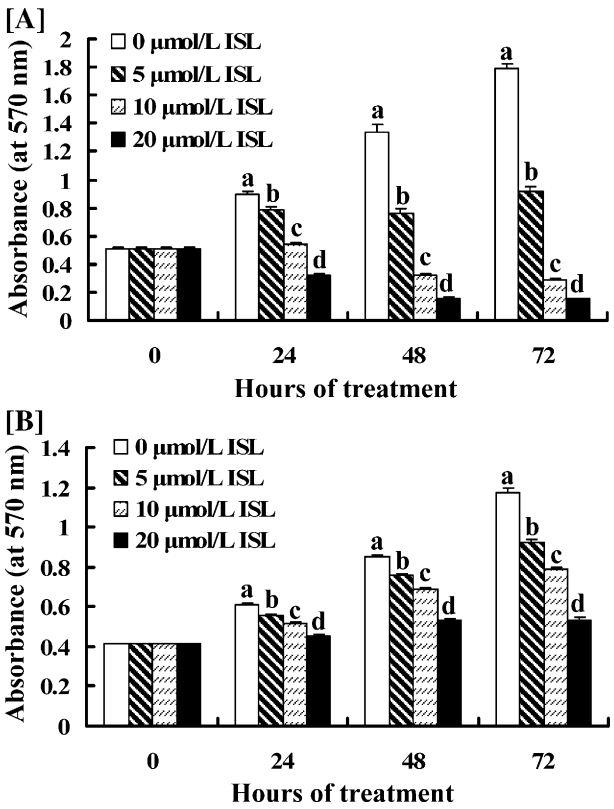

Fig. 1. Isoliquiritigenin decreases viable cell numbers in prostate cancer cells. (A) MAT-LyLu cells were plated at a density of 20,000 cells/well in 12-well plates with DMEM/F12 supplemented with 10% FBS. Forty-eight hours after plating, the monolayers were serum-starved with DMEM/F12 supplemented with 5 mg/L transferrin, 5 μg/L selenium, 2.25 mg/L glutamine and 0.1 g/L BSA (serum-free medium) for 24 h. (B) DU145 cells were plated at a density of 50,000 cells/well in 24-well plates with DMEM/F12 supplemented with 10% FBS. Twenty-four hours after plating, the monolayers were serum-starved for 24 h. Both cell lines were then incubated for 24, 48 or 72 h in serum-free medium containing 0, 5, 10 or 20 μmol/L ISL. Cell numbers were estimated by the MTT assay. Each bar represents the mean±S.E.M. ($n=3$ or 6). Means at a time without a common letter differ, $P<.05$.

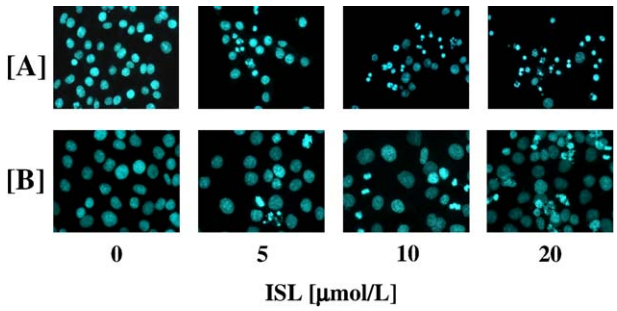

Fig. 2. Detection of apoptosis in prostate cancer cells by Hoechst Dye 33258. MAT-LyLu (A) and DU145 (B) cells were plated in chamber slides at 20,000 cells/well in DMEM/F12 supplemented with 10% FBS. After serum starvation, the monolayers were incubated for 24 h (MLL) and 48 h (DU145) in serum-free medium containing 0, 5, 10 or 20 μmol/L ISL. Cells were fixed and stained with a DNA specific dye, Hoechst 33258.

The key regulatory factors of mitochondria-mediated apoptosis are the Bcl-2 family of proteins, which can either promote cell survival or induce apoptosis [13,14]. For example, Bcl-2 appears to preserve the integrity of the mitochondrial outer membrane, thereby preventing the release of pro-apoptotic factors, whereas Bax promotes cytochrome *c* and Smac/Diablo release from mitochondria [15,16].

Isoliquiritigenin (ISL), a flavonoid with a chalcone structure, found in licorice, shallot and bean sprouts, has been reported to exhibit anticarcinogenic effects. Isoliquiritigenin has been shown to suppress 7,12-dimethylbenz[*a*]anthracene-induced and TPA-promoted skin papilloma formation [17] and to inhibit the induction of preneoplastic aberrant crypt foci in azoxymethane-treated F344 rats [18]. In vitro studies have shown that ISL induces apoptosis in hepatoma [19], gastric [20] and melanoma cancer cells [21]. However, the mechanisms by which ISL induces apoptosis in cancer cells have not been well characterized. In addition, to our knowledge, the effects of ISL on prostate cancer have not been studied in detail, with the exception of a study that reported a decreased growth and increased expression of GADD153 mRNA in LNCaP and DU145 prostate cancer cells in the presence of ISL [18].

The present study examined whether ISL induces apoptosis of prostate cancer cells and investigated the underlying mechanisms using MAT-LyLu (MLL) rat and DU145 human prostate cancer cells.

2. Materials and methods

2.1. Materials

The following antibodies were purchased from their respective sources. Cytochrome *c* and caspase-8 (BD PharMingen, Franklin Lake, NJ); cleaved caspase-9, cleaved