Food Sci. Biotechnol. Vol. 18, No. 4, pp. 1019 ~ 1021 (2009)

RESEARCH NOTE

Food Science and Biotechnology

Atractylodes japonica Rhizome Inhibits Cell Proliferation and Induces Apoptosis *in vitro*

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Abstract Antiproliferative activity of the ethanol extract of *Atractylodes japonica* rhizomes (AJEX) was investigated using methyl thiazolyl tetrazolium (MTT) assays with various cancer cell lines (HL-60, MCF-7, SK-Br-3, MDA-MB-453, HepG2, Hep3B, PC-3, LNCaP, MKN 28, MKN 45, and HT-29 cells). Gastric carcinoma cell lines were the most responsive in terms of cell proliferation. The IC_{50} of MKN 28 and MKN 45 cells were 35.98 and 27.57 μg/mL, respectively. Moreover, gastric carcinoma cells exposed to AJEX underwent apoptosis, as determined by Annexin V binding assay. Compared to respective control level, exposure to the AJEX at each IC_{50} concentration resulted in a remarkable increase in the shift of cell populations. Present results suggest that AJEX possess potential anticancer properties.

Keywords: *Atractylodes japonica*, apoptosis, cell proliferation, *in vitro*, IC_{50}

Introduction

Recently, natural phytochemicals therapies are accepted as common form of medicine, and epidemiological and experimental studies have demonstrated that traditional herbs have decreased the incidence of certain forms of cancer (1,2). The actions of herbal remedies may be due to their various biological activities, and it is now becoming widely accepted that such botanical components can make an important contribution to human health.

Dried rhizomes of *Atractylodes japonica* have been used for the treatment of stomach disorders, diuresis, pain, and arthritis in traditional pharmaceuticals of northeast Asia (3,4). Previous studies have reported that *A. japonica* have several anti-inflammatory and analgesic activity (5,6). The constituents of the rhizomes of *A. japonica* have been investigated by several researchers. Atractylons (7,8), sesquiterpenoids (9,10), and diacetyl atractylodiol (11) were isolated form the rhizomes of *A. japonica*. Molecules such as atractylon and sesquiterpenoids are used an index molecule of *A. japonica*. Previous reports have been suggested the potential therapeutic value of atractylon and sesquiterpenoids. Atractylon and sesquiterpenoids showed the significant antiproliferative effect in several cancer cells such as leukemia, melanoma, and cervical carcinoma (12,13).

In this study, anticancer effects of ethanol extract of *A. japonica* rhizomes (AJEX) on several representative human cancer cells were investigated. In addition, because AJEX was most effective in gastric carcinoma, here we assessed AJEX could induce the apoptosis of in human gastric carcinoma MKN28 and MKN45 cells.

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Received May 28, 2008; Revised March 17, 2009;
Accepted April 9, 2009

Materials and Methods

Cell cultures Human acute promyelocytic leukemia HL-60, breast adenocarcinoma MCF-7, SK-Br-3, and MDA-MB-453, hepatocellular carcinoma HepG2 and Hep3B, prostate adenocarcinoma PC-3 and LNCaP, and gastric carcinoma MKN 28, MKN 45, and colon adenocarcinoma HT-29 cells were used in the present study. These cells were purchased from the Korean Cell Line Bank (KCLB, Korea). Each cells was routinely maintained in either RPMI 1640 or Dulbecco's modified Eagle's medium (DMEM) (Invitrogen [Molecular Probes], Gibco, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (FBS) and antibiotics (50 U/mL of penicillin and 50 μg/mL streptomycin, Gibco) at 37°C in a humidified atmosphere containing 5% CO_2. Each cell lines were plated at density of $0.8\text{-}1.2\times10^6$ and $1.5\text{-}2.0\times10^3$ cells/well on 6- and 96-well plate for cell proliferation assay and apoptosis detection, respectively. After attachment, fresh medium containing AJEX at various concentrations was replaced and incubated for 24 hr.

Preparation of *A. japonica* Koidz rhizomes extract *A. japonica* was identified by Dr. HS Park (Plant Resources Research Institute, Seoul, Korea) in the provenance (Jecheon, Korea), and after that dried rhizomes of *A. japonica* were purchased through a Kyung-Dong drugstore (Seoul, Korea). In addition, *A. japonica* is raised in the herbarium of Duksung Women's University (Seoul, Korea). Ethanol extracts from *A. japonica* rhizomes (AJEX) were prepared as followed; briefly, dried rhizomes of *A. japonica* were extracted with 95% ethanol (250 g of dried materials/2,000 mL solution). The yield of the dehydrated powder among the primary net dry weight plant was about 10%(w/w). This dehydrated powder was diluted in dimethyl sulfoxide (DMSO) to 10 mg/mL just before use. The collected ethanol supernatant was evaporated in a rotary evaporator (NYC-2000; Eyela, Tokyo, Japan) under reduced pressure. In addition, the remaining ethanol was dried in desiccators with a high vacuum pump (W2v40;

Woosung Automa Co., Ltd., Gyeonggi, Korea).

Cell viability assay The inhibitory effect of the AJEX on cell proliferation was determined by the methyl thiazolyl tetrazolium (MTT) assay. The cells were treated with AJEX ranging from 1 to 125 μg/mL and incubated for 24 hr and added to MTT. Four hr later, DMSO was added to each well to dissolve the resulting formazan crystals and then absorbance was recorded at 490 nm in a microplate reader (SpectraMax Plus; Molecular Devices Corp., Sunnyvale, CA, USA). The value of IC_{50} (i.e., the concentration of the extract required to inhibit cancer cell proliferation by 50% of the control level, which is each cells treated with only compound solvent) was estimated from the plot.

Apoptosis detection assay Annexin V-FITC apoptosis kit (BD ApoAlert™; BD Biosciences Clontech, San Jose, CA, USA) was used to apoptosis detection. Cells were trypsinized, washed twice in ice-cold phosphate buffered saline (PBS), and resuspended in 500 μL binding buffer (Sigma-Aldrich, St. Louis, MO, USA). Annexin V and propidium iodide solution were added to the cell preparations and incubated for 25 min in the dark. Binding buffer (400 μL) was then added to each tube and the samples were analyzed by a FACS Calibur Instrument (BD Biosciences Clontech) equipped with CellQuest 3.3 software.

Results and Discussion

Antiproliferative activity of AJEX in several cancer cell lines The present study was designed to investigate the anticancer capacity of ethanol extracts from *A. japonica* rhizomes (AJEX). First, the antiproliferative activity of AJEX was investigated against 6 distinct cancer cell models representative of the most frequent solid cancers such as breast cancer, hepatoma, leukemia, prostate cancer, stomach cancer, and colon cancer. Antiproliferative activity of AJEX was investigated in ten human cancer cells (HL-60, MCF-7, SK-Br-3, MDA-MB-453, HepG2, Hep 3B, PC-3, LNCaP, MKN 28, MKN 45, and HT-29 cells) exposed to AJEX at concentrations ranging from 1 to 125 μg/mL for 24 hr.

Table 1 shows that gastric carcinoma cells were the most sensitive, while hepatoma cells were the most resistant cancer cells in terms of cell growth. AJEX exhibited active antiproliferative effects against MKN 28 and MKN 45 cells with the IC_{50} values of 35.98 and 27.57 μg/mL, respectively. Moreover, AJEX showed antiproliferative activity in both human gastric carcinoma MKN 28 and MKN 45 cells as a dose-dependent manner (Fig. 1A). This result consistently supports the notion that AJEX may contribute to stomach-associated disorders. This is very encouraging result, because gastric cancer is the second most common cause cancer deaths worldwide. According to registered death data by the Korea National Statistical Office in 2007, gastric cancer is also a highly prevalent malignant tumor in Korea, comprising 16.35% of all cancer sites (14). Therefore, we focused on the anticancer activity of AJEX in human gastric carcinoma MKN 28 and MKN 45 cells.

Table 1. IC_{50} value of ethanol extract of *Atractylodes japonica* rhizomes in various cancer cell lines

Group	Cell line	IC_{50} (μg/mL)[1)]
		AJEX
Breast adenocarcinoma	MCF-7	77.54
	MDA-MB-453	62.47
	SK-BR-3	72.06
Hepatocellular carcinoma	Hep3B	78.52
	HepG2	81.14
Leukemia	HL60	44.52
Prostate adenocarcinoma	PC-3	67.94
	LNCaP	71.25
Gastric carcinoma	MKN 28	35.98
	MKN 45	27.57
Colon adenocarcinoma	HT 29	36.85

[1)]Reductions of cell density were plotted against different concentrations of extract (1-125 μg/mL), and the value of IC_{50} was estimated from the plot. AJEX, ethanol extracts from *A. japonica* rhizomes.

Proapoptotic activity of AJEX in human gastric carcinoma To verify that anticancer activity of AJEX, the flow cytometric annexin V-based analysis was has been applied in human gastric carcinoma MKN 28 and MKN 45 cells. Apoptosis is an important series of events that leads to programmed cell death and is essential for the development and homeostasis of tissues. The potential mechanisms for the apoptotic process involve the balance between apoptosis induction and apoptosis inhibition factors. Recently, the therapeutic pro-apoptotic agents have been proposed as one of the major strategies of cancer chemotherapy (15,16).

After exposing MKN 28 and MKN 45 cells to AJEX at each IC_{50} concentration, apoptosis induction was remarkably observed (Fig. 1B). Compared with respectively control level, apoptotic cell population was increased by 40.39 and 52.66% in MKN28 and MKN45 cell, respectively. It has been reported that some of plant extracts act through the induction of apoptosis in various cancer cells (17,18). In addition, previous studies have demonstrated that some of the herbs from the Atractylodes family have physiological effects (5,6,12); *Atractylodes lancea* could improve the gastric disorders such as the delay of gastric emptying and ulcer (19,20), and *Atractylodes macrocephala* extracts have been reported to have anti-inflammatory activity (21,22).

The present results show that the antiproliferative effect of AJEX resulted in apoptosis induction, which are consistent with the notion that AJEX has been used for the treatment of stomach-related disorders. Recently, natural phytochemicals in medical plants are being explored for biological activity such as antiproliferative and proapoptotic activity to attempt as a chemoprevention or chemotherapeutics (23).

Based on our results, this study suggests that AJEX may be useful for gastric cancer chemotherapy, although the active compounds in AJCX have yet to be identified. Therefore, in future, identifying the compounds in the extracts of *A. japonica* rhizomes would be of great interest in the treatment of gastric carcinoma.

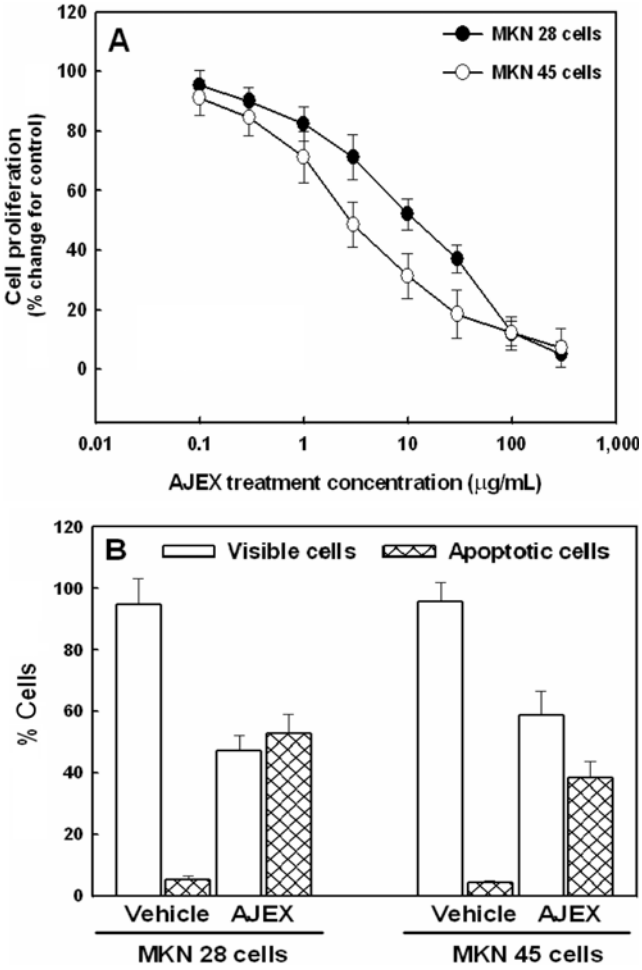

Fig. 1. Ethanol extract of *A. japonica* rhizomes inhibited proliferation (A) and induced the apoptosis (B) in human gastric carcinoma MKN28 and MKN45 cells. Cells were exposed to AJEX at each IC_{50} concentration. Data are expressed as mean±SD from 3 independent experiments.

Acknowledgments

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, KRF-2005-005-J13001).

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