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Cruciferous phytoalexins: antiproliferative effects in T-Jurkat leukemic cells

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Abstract

We tested antiproliferative activity of selected cruciferous phytoalexins including brassinin, 1-methoxybrassinin, (±)-spirobrassin, (±)-1-methoxyspirobrassinin and (±)-1-methoxyspirobrassinol, in leukemic Jurkat cell. The most effective of the tested phytoalexins was 1-methoxybrassinin with IC_{50} 10 μmol l^{-1}. However, significant effect of all phytoalexines was also determined at concentration 1 μmol l^{-1}. In 1-methoxybrassinin-treated Jurkat cells, we found significant increase in the fraction of cells with a sub-G_0/G_1 DNA content, which is considered to be a marker of cell death by apoptosis. Apoptosis was also confirmed by the annexin V staining.

In summary, 1-methoxybrassinin exerted potent antiproliferative activity probably due to cell cycle arrest and apoptosis induction.

Keywords: Cruciferous phytoalexins; Antiproliferative effects; Apoptosis; Leukemia

1. Introduction

Edible plant matter, such as fruit, vegetables and cereals contain many microconstituents that are now recognized as being biologically active. In the recent decades, considerable attention has been directed on cancer prevention by natural products [1,2]. Some epidemiological studies indicated that diet may play a significant role in controlling the risk of developing cancers and that increased consumption of fruits and vegetables reduces cancer incidence in humans [3–5]. Natural compounds may act as potential chemopreventive and chemotherapeutic agents by different mechanisms including inhibition of uncontrolled cell growth, apoptosis induction or promoting the differentiation of cancer cells [6–8].

A group of vegetables with considerable anticancer properties are the plants from *Cruciferous* family. Epidemiological studies have shown that a diet rich in cruciferous vegetables such as broccoli, Brussels sprouts, cabbage and cauliflower, can lower the risk of various cancers [9–13]. The major active compounds in cruciferous vegetables, indole-3-carbinol and sulforaphane exhibit promising cancer protective properties in vitro and in vivo [14–17]. It is believed that chemopreventive effects of above-mentioned compounds are due to their ability to influence the metabolism of carcinogens [18–20].

Another indole-based group of compounds naturally occurring in cruciferous vegetables are phytoalexins. These secondary metabolites, which are synthesized de novo in response to diverse forms of stress, including fungal infection, are part of the plants' chemical and biochemical defense mechanisms [21]. Moreover, there are few indices that phytoalexins may also act as chemopreventive or antiproliferative agents.

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In the last decade, Mehta et al. [22–24] studied the chemopreventive effect of brassinin and related compounds in in vitro model of mammary carcinogenesis. They found that brassinin, cyclobrassinin and spirobrassinin inhibited the formation of dimethylbenz(a)anthracene-induced mammary lesions in a dose-dependent manner.

Later, the antiproliferative effect of indole phytoalexins brassinin and spirobrassinin in mouse leukemia L1210 and melanoma B16 cells was also documented. The highest cytotoxic effect was induced by brassinin, which at concentration 100 $\mu mol\,l^{-1}$ reduced the cell growth of L1210 and B16 by 35% of the solvent control [25].

The present study was conducted to examine the effects of selected cruciferous phytoalexins on proliferation and cell death in the human leukemic Jurkat cell line.

2. Materials and methods

2.1. Reagents and drugs

MTT–3-(dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was from (Sigma-Aldrich Chemie, Steinheim, Germany). Tested chemicals: brassinin (1), 1-methoxybrassinin (2), (±)-spirobrassinin (3), (±)-1-methoxyspirobrassinin (4) and (±)-1-methoxyspirobrassinol (5) (Fig. 1), were synthetised by Kutchy et al. [26]. Cycle TEST^{TM} PLUS DNA Reagent Kit, annexin V-FITC and propidium iodide were purchased from Becton Dickinson, USA.

2.2. Cell culture

Jurkat cells (human acute T-lymphoblastic leukemia cells) were kindly provided by Dr. M. Hajdúch (Olomouc, Czech Republic). Cells were maintained in RPMI 1640 medium with Glutamax-I supplemented with 10% foetal calf serum, penicillin (100 IU ml^{-1}) and streptomycin (100 $\mu g\,ml^{-1}$) (all from Invitrogen, UK), in the atmosphere 5% CO_2 in humidified air at 37 °C. Cell viability, estimated by trypan blue exclusion, was greater than 95% before each experiment.

2.3. Assessment of cytotoxicity by MTT assay

Cytotoxic effect of the tested compounds was studied by using colorimetric microculture assay with the MTT end-point. The amount of MTT reduced to formazan is proportional to the number of viable cells [27]. Briefly, 8×10^4 cells were plated per well in 96-well polystyrene microplates (Sarstedt, Germany) in the culture medium containing the tested chemicals at final concentrations between 10^{-4} and 10^{-6} mol l^{-1}. After 72 h incubation, 10 μl of MTT (5 mg ml^{-1}) were added in each well. After additional 4 h, during which insoluble formazan was produced, 100 μl of 10% sodium dodecylsulphate were added in each well and another 12 h were allowed the formazan to be dissolved. The absorbance was measured at 540 nm using the automated MRX microplate reader (Dynatech Laboratories UK). Absorbance of control wells was taken as 100%, and the results were expressed as a percent of control.

2.4. Cell cycle analysis

Cell cycle distribution in cells treated with the tested agents was analyzed by propidium iodide DNA staining using Cycle TEST^{TM} PLUS DNA Reagent Kit (Becton Dickinson, USA). Briefly, 5×10^5 Jurkat cells were treated with phytoalexins at concentration 100 $\mu mol\,l^{-1}$ for 24, 48 and 72 h. After treatment, cells were harvested, washed thrice in citrate buffer and further processed and stained according to the manufacturer's instructions. Then, within one hour after staining, data acquisition was performed in a FACS Vantage SE flow cytometer using the CellQuest Pro software (Becton Dickinson, USA), information being stored for 5×10^4 events per sample. Propidium iodide fluorescence was detected in the pulse-processed FL3 channel (630/22 nm band pass filter). The data were analyzed using Win MDI software

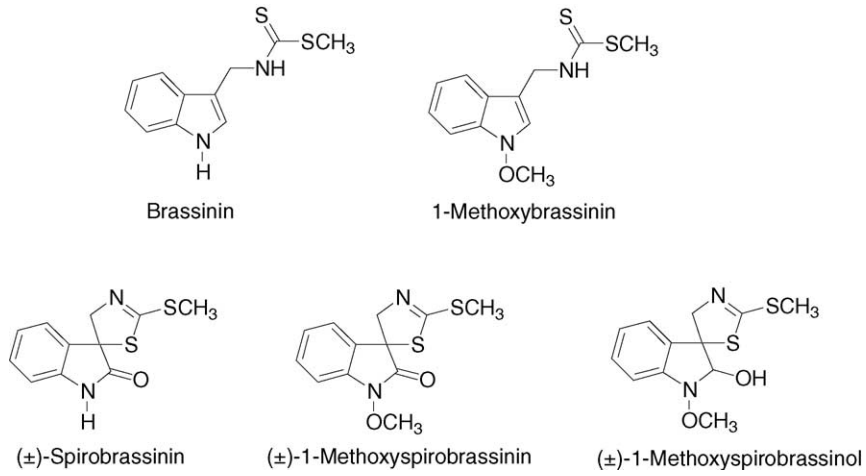

Fig. 1. The cruciferous phytoalexins used in this study.