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Antiproliferative effect of 1-methoxybrassinin

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Introduction

Several epidemiologic studies suggest that consumption of cruciferous vegetables may be particularly effective in reducing cancer risk at several organ sites[1, 2]. Indole phytoalexins represent a specific group of phytoalexins synthesized by plants of the family *Cruciferae*.

Phytoalexins are anti-microbial secondary metabolites of low molecular weight produced by plants *de novo* after exposure to biological, physical or chemical stress[3]. These substances are usually produced in small quantities. Chemical synthesis can provide access to reasonable amounts of phytoalexins that are necessary to evaluate their biological activities. Indole phytoalexins have been reported to exhibit several biological activities, including chemopreventive[4] antiproliferative, antifungal[5], antiprotozoal[6] and anticarcinogenic[7] activities. The unique structural feature of the majority of indole phytoalexins is the presence of an indole ring and side chain or another heterocycle, containing a nitrogen atom and one or two sulpfur atoms[8]. Until now 44 indole phytoalexins, i.e. metabolites have been isolated and their structure elucidated[9]. 1-methoxybrassinin, brassinin and cyclobrassinin were the first cruciferous phytoalexins, isolated from the Chinese cabbage after infection with the bacterium *Pseudomonas cichorii*[10]. These natural substances have been suggested as a potential anti-tumor agents but little is known about their inhibitory mechanism on the growth of cancer cells.

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The present study was conducted to examine the effects of 1-methoxybrassinin on cell proliferation in the different human cancer cell lines.

Experimental methods

Tested compound

The synthesis of 1-methoxybrassinin was described in the previous studie: Budovska et al., 2013.[11]

Cell culture

The human cancer cell lines: DMS114 – small cell lung cancer**,** H1437 – adenocarcinoma; non-small cell lung cancer, MDA-MB-231 – breast carcinoma cell line, ZR-75-1 – breast carcinoma cell line were cultured in RPMI 1640 medium (PAA Laboratories, Pasching, Austria) and Caco-2 (colorectal carcinoma) was maintained in growth medium consisting of high glucose Dulbecco's Modified Eagle Medium (Invitrogen, USA). Both media were supplemented with a 10% fetal bovine serum (FBS), penicillin (100 IU/ml) and streptomycin (100 g/ml) (all from Invitrogen, USA) in a atmosphere containing 5% CO_2 in humidified air at 37 °C. Cell viability, estimated by trypan blue exclusion, was greater than 95% before each experiment.

MTS assay

The effect of compound on cell proliferation was determined using the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium)] test. Cells were seeded in 96 wells plates at a density of 5×10^3 cells/well. 48 hours after cell seeding different concentrations (10^{-4}–10^{-6} μM) of compound were added and the plates incubated at 37 °C for additional 72 hours. At the end of the treatment period, MTS reagent (Promega) was added to each well and the plates were icubated at 37 °C for 4 hours in 5% CO_2. Cell proliferation was evaluated by measuring the absorbance at 490 nm using an infinite M200 Microplate Reader Tecan. A nonlinear regression methods was used to calculate IC values (concentrations required to produce 50% growth inhibition).

Table 1. The IC_{50} (μM) of 1-methoxybrassinin in different cancer cell lines after 72 h incubation. Data is presented as a mean ± S.D. of three independent experimental determinations performed in triplicate.

Human cancer cell lines	MDA-MB-231	ZR-75-1	DMS114	H1437	Caco-2
IC_{50} μM	31.3 (± 1.6)	32.9 (± 2.3)	38.5 (± 2.4)	77.5 (± 3.1)	8.2 (± 1.2)

5-Bromo-20-deoxyuridine (BrdU) cell proliferation assay

Cell proliferation activity was directly monitored by quantification of BrdU incorporated into the genomic DNA during cell growth. DNA synthesis was assessed using colorimetric cell proliferation ELISA assay (Roche Diagnostics GmbH, Mannheim, Germany) following the vendor's protocol.

Colony formation assay

To determine colony formation, cells were seeded in 6-well plates (5×10^3 cells/well) and allowed to adhere for 24 h before treatment. A culture medium containing variable concentrations of the tested compound was added to cells and incubated for 10 days to allow colony formation. Colonies were then fixed in 4% formaldehyde at room temperature for 30 min and stained with 0.01% crystal violet. The crystal violet stain was then extracted with 10% acetic acid for 60 min and read at 540 nm. Cell survival at each drug concentration was expressed as a percentage of survival of controls.

Statictical analysis

Results are expressed as mean ± SD. Statistical analyses of the data were performed using standard procedures, with one-way ANOVA followed by the Bonferroni multiple comparisons test. Differences were considered significant when P values were smaller than 0.05.

Results and discussion

The antiproliferative effect of 1-methoxybrassinin on cancer cells was evaluated by MTS assay using different concentrations. As shown in Table 1, 1-methoxybrassinin suppressed cell proliferation with IC_{50} values ranging from 8.2 to > 77 μM. This compound exhibited the most significant inhibitory effects on the growth of Caco-2 cells (IC_{50} = 8.2 (± 1.2) μM). Based on these results, further experiments were performed with 1-methoxybrassinin on the most sensitive cancer cell line, Caco-2. Results of our previous study[12] show a similar inhibitory effect of 1-methoxybrassinin on growth of leukemic Jurkat cell. We have shown that among five phytoalexins examined, this potential substance exhibited a significantly stronger growth inhibitory effect (IC_{50} = 10 μM) in Jurkat cells than other compounds used.

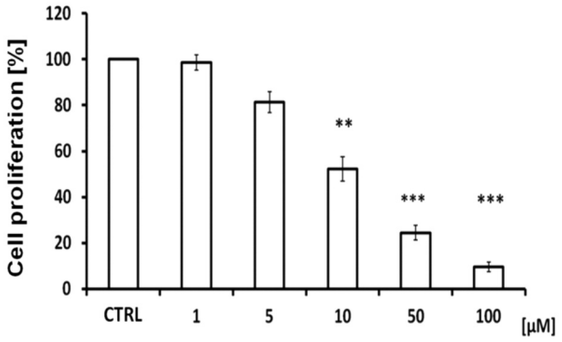

Fig. 1. Effects of 1-methoxybrassinin on the proliferation of Caco-2 cells, as examined by BrdU incorporation
**** p < 0.001, ** p < 0,01, * p < 0.05 verzus control (untreated)*

To confirm the potential antiproliferative effect of 1-methoxybrassinin, the BrdU proliferation assay was used. The magnitude of the absorbance for the developed colour is proportional to the quantity of BrdU incorporated into cells, which is a direct indication of cell proliferation. As shown in Fig. 1, 1-methoxybrassinin at concentrations of 100, 50 and 10 μM significantly decreased BrdU incorporation compared with the control (approximately 90%, 75% and 47% respectively) ($p < 0.001$; $p < 0.05$). Antiproliferative effect was not observed at the concentration of 1 μM.

To evaluate the effect of tested compound on Caco-2 colony-forming ability, a colony formation assay was performed. As shown in Figure 2, 1-methoxybrassinin again inhibits the proliferation of Caco-2 cells in a concentration-dependent manner.

Izutani et al., (2012)[7] have described the ability of brassinin inhibit the growth of colorectal cancer cells by blocking the cell cycle in the G1 phase by over-expression

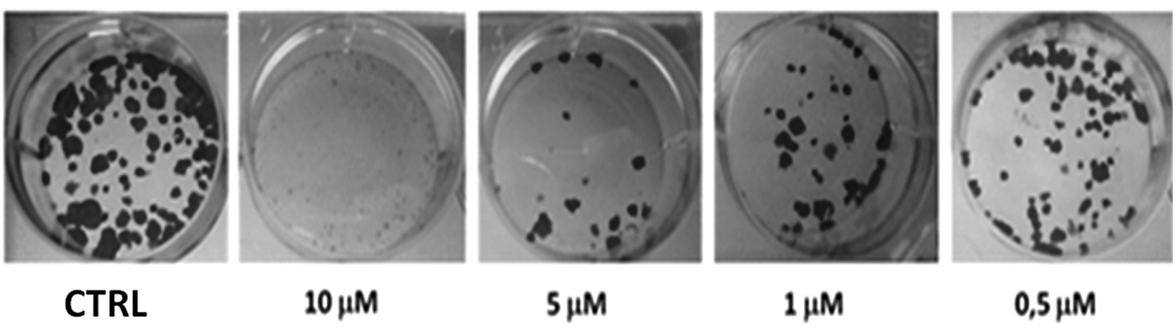

Fig. 2. Inhibition of clonogenic growth of Caco-2 cells after10 days incubation with 1-methoxybrassinin (0.5–10 μM)

of the p21 proteins and p27 and inhibition of PI3K/Akt signaling pathway. Recent data suggest possible interference of brassinin with the PI3K/Akt/mTOR/S6K1 signaling pathways. Activation of the PI3K/Akt/mTOR/S6K1 is closely linked with the development of prostate cancer metastasis and angiogenesis.

Conclusions

In conclusion, our results indicate that 1-methoxybrassinin is a potential natural inhibitor of proliferation of colorectal cancer cells. Further experiments are necessary to clarify the mechanism of action of 1-methoxybrassinin.

Acknowledgments

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Conflicts of interest: none.

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