Investigation of the antiproliferative effect of natural sesquiterpene lactones on human cancer cell lines

Judit Molnár1, Ildikó Lajter2, Zsuzsanna Hajdú2, Thomas Szekeres3, Philipp Saiko3, Judit Hohmann2, István Zupkó1,
1Department of Pharmacodynamics and Biopharmacy, University of Szeged, Hungary
2Department of Pharmacognosy, University of Szeged, Hungary
3Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Vienna

AIM OF THE STUDY

Plant extracts and natural products play a crucial role in the research of novel antineoplastics agents. The aim of the present study was to investigate the antiproliferative effect of five sesquiterpene lactones (1-5, Fig. 1) isolated from Asteraceae species (Artemisia asiatica L. and Onopordum acanthium L.) in vitro using human cancer cell lines (HeLa, A431, MCF7, HL-60). The most effective compound were selected for additional experiments in order to characterize the possible mechanism of action.

METHODS

MTT assay

Human adherent cell lines (HeLa, A431 and MCF7 from cervix, skin and breast adenocarcinoma, respectively) were exposed to the tested compounds for 72 hours and then assayed by MTT and IC50 values were determined.

Antiproliferation assay

HL-60 cells were exposed to the compounds for 24, 48 and 72 hours and counted by microcellcounter and IC50 values were calculated.

Cell cycle analysis

HL-60 cells were exposed to the most effective agent for 24 hours. DNA was stained with propidium iodide (10 μg/mL) in the presence of RNAase (50 μg/mL). The samples were analyzed by CyFlow and the cell cycle distributions were calculated by ModFit LT 3.3.

Hoechst 33258 propidium iodide (HO-Pi) double-staining

HL-60 cells treated with the test substance for 24 hours and then staining solution was added (HO and Pi: 5 and 2 μg/mL, respectively). After washing cells were viewed and photographed with a Nikon Eclipse equipped with a epifluorescence attachment and a QCapture CCD camera. This staining allowed the identification of intact, early apoptotic, late apoptotic and necrotic cells. HO permeates all cells and makes the nuclei blue. Pi is taken up by cells only when the cytoplasmatic membrane integrity has been lost staining the nucleus red.

Caspase-3 activity assay

A fluorimetric kit (Sigma-Aldrich) was used to determine the activity of caspase-3 in HL-60 cells after 24 hours of treatment.

Calculations and statistical evaluations were performed with GraphPad Prism 4.0.

CONCLUSIONS

1. The tested sesquiterpene lactones exerted moderate antiproliferative action on human adherent cell lines (HeLa, A431, MCF7; Fig. 1).
2. These compounds have more potent action on HL-60 promyelocytic leukemia cell line (Fig. 2).
3. Compound 5 caused a cell cycle arrest at G2/M phase (Fig. 3).
4. Apoptosis inducing capacity of 5 has been evidenced by an increase in subG1 phase (Fig. 3).
5. Morphological findings also support the apoptosis induced by the 5 (Fig. 4). No significant caspase-3 activation has been detected after a 24 h treatment.

REFERENCES


Figure 1. Chemical structures of the tested natural products and their calculated IC50 values.

Figure 2. Concentration-response curves of compound 5 on HL-60 cell line after 24 h, 48 h and 72 h incubation periods.

Figure 3. Effect of compound 5 on cell cycle phase distribution of HL-60 cells after incubation for 24 h. * and ** indicates p<0.05 and p<0.01 as compared with the control cells, respectively.

Figure 4. Fluorescent pictures of the effect of compound 5 on HL-60 cells after incubation for 24 h.