Mgr Barbara Gieroba
Zakład Biologii Komórki,
Instytut Biologii i Biochemii,
Wydział Biologii i Biotechnologii,
Uniwersytet Marii Curie-Skłodowskiej w Lublinie

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Organizacja molekularna oraz aktywność przeciwnowotworowa ksantohumolu w odniesieniu do modelowych błon lipidowych i wybranych linii komórek nowotworowych

(Molecular organization and anticancer activity of xanthohumol in relation to model lipid membranes and selected cancer cell lines)

Abstract

Flavonoid xanthohumol (XN), which is the main polyphenol present in the female inflorescences of hop cones (*Humulus lupulus* L.), has received much attention in recent years due to its numerous reported health-promoting properties. It shows, among others, antitumor, antioxidative, antiangiogenic, antiinflammatory, antimicrobial and neuroprotective activity. It also affects favorably the prevention from diseases that accompany the metabolic syndrome, such as obesity, diabetes and hypertension, which are significant civilization issues. Based on these reports, in hereby dissertation the cytotoxic effect of XN towards two cancer cell lines: prostate cancer PC-3 and breast cancer T47D was investigated and then it was determined whether and how it induces cell death – apoptosis or necrosis. The impact of XN on normal cells – human dermal fibroblast line NHDF was also evaluated.

The effect of XN on cells was determined spectrophotometrically using MTT and NR assays, on the basis of which the half maximal inhibitory concentration (IC_{50}) value was determined as well as the result of morphological evaluation of cells stained by the MGG and acridine orange/ethidium bromide method, which collectively with cytometric analysis of Annexin V/FITC labelled cells allowed for the determination of the population of apoptotic and necrotic cells. The degeneration of cells was confirmed by Fourier transform infrared spectroscopy (FTIR-ATR). In the first part of the research it was observed that:

- Cells of both cancer lines showed susceptibility to XN, manifested by the occurence of the cytotoxic effect (the IC_{50} value for the PC-3 cell line in the MTT and NR assays was respectively 33,28 μM and 15,20 μM, for the T47D cell line 32,08 μM and 5,78 μM). The T47D breast cancer cell line was slightly more sensitive to XN than the PC-3 prostate cancer cell line;
- Cell sensitivity to XN showed a dependence on its dose and time of incubation;
- The decrease in cell viability occured due to the induction of both apoptosis and necrosis by XN;
- XN caused changes in lipids, proteins and nucleic acids, affecting their molecular organization;
- The least prone to the action of XN was normal human fibroblasts cell line.

Another aim of this doctoral thesis was to study the interaction of XN with lipids using the Langmuir monolayers technique and Brewster angle microscopy (BAM) in binary (with DPPC, POPC, SM, Chol and GM_1) and in ternary model systems (with DPPC and Chol in mutual ratio 1:1 – a model of normal cell membrane, SM and Chol in 1:1 proportion – a model of lipid raft, SM and Chol in mutual ratio 7:3 – a model of breast cancer cell membrane and POPC and Chol in a 7,5: 2,5 ratio - a model of prostate cancer cell membrane). On the basis of the obtained π-A isotherms and BAM images, a thermodynamic analysis of mutual interactions was performed. A series of experiments involving incorporation XN into monolayers formed from lipids in order to determine the adsorption kinetics was also carried out. The observations are as follows:

- XN showed stronger interactions and mutual miscibility in model systems mimicking pathological membranes than in model systems reflecting normal membranes;
- Strong interactions of XN with Cholesterol were found;
- XN showed complete miscibility with GM1, which is known to be overexpressed in cancer cell membranes;
- Significant increases in surface pressure after the injection of XN under lipid monolayers indicate a strong affinity of this chalcone to membrane lipids.

Considering the high selectivity of XN towards cancer cells, it may constitute a potential therapeutic in oncological treatment in the future.