PLANT-DERIVED COMPOUNDS AS EFFECTIVE MODULATORS OF THE LIPID MEMBRANE PROPERTIES AND THE ACTIVITY OF MEMBRANE TRANSPORTERS

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The effect of plant-derived polyphenols that belong to different groups of compounds (flavonoids, stilbenes) on membrane lipid structure and transport activity of the membrane proteins playing important role in cancer cells proliferation and apoptosis and in anticancer drug resistance was studied. The influence of flavonoids on yeast multidrug transporters was also investigated. The effect of polyphenols on biophysical properties of lipid model membranes was observed using fluorescence and EPR spectroscopy, and microcalorimetry. The effect of flavanolignan, silybin (Wesołowska *et al*. 2007) and stilbene derivatives (e.g. resveratrol, piceatannol) on lipid membrane properties were discussed. Silybin is the main flavanolignan extracted from the seeds and fruits of *Sylibum marianum* (milk thistle) and resveratrol is 3,4',5-trihydroxystilbene found in grapes. Both compounds exhibit various biological activities including anticancer properties. The influence of plant polyphenols on integral membrane proteins can be mediated at least in part by observed non-specific membrane effects.

The most important multidrug resistance (MDR) transporters in tumour cells are P-gp (P-glycoprotein) and MRP1 (multidrug resistance associated protein, ABCC1). MRP1 is also present in different kinds of normal cells. Its expression is well established in erythrocytes. Fluorescent substrates of drug transporters are often used to study their activity. Transport activity of MRP1 was measured in human erythrocytes using the functional assay based on the efflux of fluorescent probe BCECF, a derivative of carboxyfluorescein. The molecular mechanism that regulates transport of different MRP1 substrates across cell membranes is not well understood. Genistein was the first flavonoid found to be a potent inhibitor of transport activity of MRP1. In these studies the ability of various kinds of naturally occurring flavonoids (e.g. flavones, isoflavones, flavanolignan) to modulate a transport activity of MRP1 was investigated. Most of the studied flavonoids showed strong or moderate ability to inhibit transport activity of MRP1. Among studied flavonoids two compounds occurred to be especially potent inhibitors: silybin (flavananolignan) and morin (flavonol) as it might be concluded from obtained values of the inhibitory concentration IC_{50} and the maximal inhibition (Łania-Pietrzak *et al*., 2005). Licoisoflavones, genistein (isoflavones), apigenin (flavone), naringenin (flavanone) and aromadendrin (flavanol) also appeared to be good inhibitors of MRP1 transport activity in erythrocytes. Taking into account the important role of MRP1 in normal tissues and in multidrug resistance of cancer cells further studies are needed to elucidate the molecular mechanism of inhibition of MRP1-mediated drug transport by flavonoids.

In recent years an important role of ion channels in cancer has emerged. Ion channels may affect pathways regulating cell proliferation and apoptosis. It was shown that particularly potassium channels participate in cancer development and apoptosis. An expression of voltage-dependent Kv1.3 channels is up-regulated for example in breast or colon cancer and in human gliomas. Kv1.3 channels are also involved in apoptosis of Jurkat T lymphocytes. K^+ channel blockers inhibit cell proliferation in different cell lines.

Voltage-dependent potassium channels blocking effect of MDR modulators was studied by patch-clamp technique. The ability of some natural flavonoids to inhibit transport activity of MRP1 was compared with their effect on voltage-gated potassium channels Kv1.3. Whole-cell potassium currents were recorded. Isoflavone genistein, known MRP1 inhibitor occurred to be also effective potassium channels' blocker. Application of genistein decreased the whole-cell potassium currents in T lymphocytes in a concentration-dependent manner. In the same range of concentrations as used in experiments on MRP1 transport activity, genistein reduced the activity of Kv1.3 channels to less than a half of its control value. The inhibition of current was correlated with a significant decrease of current activation rate. Inhibitory effect of genistein was reversible (Teisseyre & Michalak, 2005).

In recent years some stilbenes naturally occurring in plants have drawn much attention because of their differential biological activities. Resveratrol, piceatannol and their derivatives exert various biological effects by modifying multiple cellular targets. Their chemopreventive and anticancer properties seem to be particularly interesting. Resveratrol (3,4',5-trihydroxystilbene) and its analog – piceatannol (3,4,3',5'-tetrahydroxystilbene) can inhibit the growth of many cancer cell lines (Wolter *et al*., 2002). 3,5-dimethoxy analogs of resveratrol and piceatannol (pterostilbenes) are effective apoptosis inducing agents (Tolomeo *et al*., 2005). Our studies revealed that resveratrol and some its derivatives can modify activity of multidrug resistance associated protein MRP1 and Kv1.3 potassium channels.

Taking into account that some of natural MDR reversers (e.g. genistein or resveratrol) were effective inhibitors of both multidrug transporter and voltage-gated potassium channels Kv1.3 in future K^+ channels and MDR proteins could be considered as common potential target for cancer chemotherapy.

Activity of flavonoids as multidrug resistance modulators was also observed in case of yeast multidrug transporters. Our studies revealed that among flavonoids it might be found the effective agents inhibiting yeast growth and modulating multidrug transporter Cdr1p from *Candida albicans*. The studies performed also with Cdr1p mutants pointed out the two amino acid residues that might play an essential role in activity of Cdr1p.