Abstract

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Title of Doctoral Thesis: Sesquiterpenes in cancer therapy.

Chemotherapy is an important part of treatment of cancer. Currently, the use of several anticancer drugs in combination seem to be more advantageous. This combination therapy prevents drug resistance development and make it possible to lower the doses of single drugs, which prevents the side effects in non-cancerous tissues. Large pool of potential drugs for combination therapy can be found among natural compounds, for example among sesquiterpenes.

Our project was designed to evaluate the effect of *Myrica rubra* essential oil (MEO) and selected sesquiterpenes in combination cancer therapy. We focused on the potential ability of MEO and sesquiterpenes to increase the antiproliferative efficacy of classical cytostatics (doxorubicin, oxaliplatin, 5-fluorouracil). In addition, we also studied the possible interaction of sesquiterpenes with other drugs in combination therapy, which could be caused by sesquiterpene-mediated modulation of drug-metabolizing enzymes. For these purposes, a panel of various cell lines (both cancerous and non-cancerous) and primary culture of hepatocytes were used in *in vitro* experiments. Normal and tumor bearing mice were chosen as *in vivo* models.

MEO has significant antiproliferative effect in intestine cancer cell lines HTC-8, HT-29, SW480, SW620 and Caco-2. MEO was able to potentiate efficacy of DOX in cancer cells. Increase of efficacy correlated with enhanced intracellular DOX concentration and increased production of ROS. MEO has no effect on viability, intracellular DOX concentration and ROS production in primary culture of hepatocytes. Sesquiterpenes β-caryophyllene (CAR), β-caryophyllene oxide (CAO), α-humulene (HUM), *trans*-nerolidol (NER) and valencene (VAL) are main components of MEO. Their antiproliferative effects were tested in cancer cell lines, when they were used alone and in combination with selected cytostatics DOX, 5-fluorouracil, oxaliplatin. All

sesquiterpenes (except CAR) were able to increase DOX efficacy in Caco-2 cell line. Viability of hepatocytes was not affected by sesquiterpenes. Antiproliferative activity of OxPt and FU was increased, dominantly by CAO. In the next study, ovarian cancer cell lines with different sensitivity and two lymphoblast cancer cell lines, one sensitive and the other DOX completely resistant (with overexpression of ABCB1 transporter) were used. Sesquiterpenes were able to increase efficacy of DOX in all cell lines with exception of DOX resistant cell line. Nevertheless, sesquiterpenes were able to increase intracellular DOX concentration and to inhibit ABCB1 transporter in DOX-resistant cell line. *In vivo*, the use of sesquiterpenes in combination with DOX led to neither increase of DOX concentration in tumors nor size of tumors in tumor bearing mice.

In vitro study of potential inhibitory effect of sesquiterpenes on drug-metabolizing enzymes showed significant ability of sesquiterpenes to inhibit cytochrome P450 (CYP). Activities of carbonyl reducing enzymes and conjugating enzymes were not inhibited by sesquiterpenes. On the other hand, CAO and NER *in vivo* increased activities and expression of CYP2B, 3A and 2C in mouse liver and small intestine. In liver, elevated activity of aldo-ketoreductase 1C, carbonyl reductase and sulpfhotransferase were also found. Contrary, sesquiterpenes decreased NAD(P)H:quinone oxidoreductase 1 activity in small intestine.

Results from this doctoral thesis show possible beneficial effects of sesquiterpenes in cancer treatment. However, it is necessary to take into mind their ability to modulate activity of drug-metabolizing enzymes and possibility of sesquiterpene-drug interactions.