Abstract

Despite advances in cancer diagnosis and therapy, cancer is the second leading cause of death globally. The improvements of cancer treatment are the major challenge in this research. The aim of the thesis was studying of effects of two anticancer drugs ellipticine (Elli) and doxorubicin (DOX) on some cancer and healthy cell lines. Specific consideration was given to expand current knowledge about the metabolism and cytostatic effects of Elli in neuroblastoma cell lines. Another part of this study was focused on mechanisms contributing to the development of ellipticine-resistance in cancer cells and influence of histone deacetylase inhibitors on anticancer therapy was investigated. Moreover, the aim was to develop apoferritin (Apo) nanocarrier suitable for the active transport of cytostatics to cancer cells. Several essential data were found in this doctoral thesis. Anticancer efficiency of Elli depends on the CYP3A4-mediated metabolism in cancer. The CYP3A4 enzyme encapsulated into two nanoparticle forms, liposomes and Supersomes^{TM}, was tested to activate ellipticine to its reactive species forming covalent DNA adducts. The formation of adducts seems to be dependent on concentrations of CYP3A4 in nanoparticle systems. A higher effectiveness of CYP3A4 in Supersomes^{TM} than in liposomes to form ellipticine-DNA adducts was caused by the presence of all spectrum of membrane-making lipids, proteins and cytochrome b_5. Nanoparticle forms of CYP3A4 seem to be suitable for delivery of the enzyme to cancer cells. The results found in this study demonstrate that sequestration of Elli into lysosomes of neuroblastoma cells is one of the mechanisms contributing to the development of Elli-resistance in these cells. This sequestration resulted in lower cytoplasmic concentrations of Elli and less nuclear accumulation and therefore also lower toxic effects to these cells. We demonstrated that this resistance is dependent on upregulation of the vacuolar (V)-ATPase. Pretreatment with V-ATPase inhibitors decreased sequestration of Elli in lysosomes and enhanced the cytotoxicity of this anticancer drug. The influence of histone deacetylase inhibitor valproate (VPA) combined with Elli on neuroblastoma cells was investigated. The synergism of their efficacy was detected only after either simultaneous exposure to these drugs or after pretreatment of cells with Elli before VPA. VPA increases the acetylation of histones H3 and H4 that is important to improve binding of Elli to DNA leading to the formation of covalent adducts with DNA which is the most important mechanism of anticancer effect of Elli. One of the approaches to decrease the adverse effects of drugs is their encapsulation inside a suitable nanocarrier, Apo, allowing for a targeted delivery to tumor tissue whereas avoiding healthy cells. Apo is selectively recognized by membrane receptors SCARA5 and TfR1, highly expressed in many cancer cells. Elli either free or

released from Apo was concentrated in the nuclei of neuroblastoma cells. In fibroblasts the higher amounts of Elli were sequestrated in lysosomes that resulted in the lower cytotoxic effect of cytostatic. In addition, to enhance the nanoparticle specificity, targeting antibodies can be bind to Apo. Herein, we describe a novel approach for targeting of Apo (encapsulating DOX) to prostate cancer using antibodies against prostate specific membrane antigen that is overexpressed in prostate cancer cells. Prepared nanocarrier specifically targeted cancer cells. Modification of its surface reduced toxic effects of DOX in healthy cells.

Key words: apoferritin, chemoresistance, cytochromes P450, doxorubicin, ellipticine, histone deacetylase inhibitors, cancer cell lines, prostate cancer, nanoparticles, neuroblastoma

(In Czech)