Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Biochemical Sciences

Title, Name, Surname of candidate: Lenka Vildová
Title, Name, Surname of tutor: Doc. Ing. Barbora Szotáková, Ph.D.
Title, Name, Surname of tutor-specialist: Mgr. Martina Gavelová, Ph.D.
Title of a diploma work: **Influence of selected cytostatic drugs on activity of cytosolic carbonyl reducing enzymes in MCF-7 cell line**

A major problem in cytostatic treatment of malignant tumors is the development of drug-resistance. One of the potential mechanisms of resistance is induction of drug-inactivating enzymes. The aim of the present study was to evaluate the effects of short-term exposure of MCF-7 cells to doxorubicin and oracin on the activities and expression of selected carbonyl reducing enzymes. Carbonyl reduction of these cytostatic drugs leads to 13-doxorubicinol (doxol) and 11-dihydrooracin (DHO), the major metabolites with lower antineoplastic potency compared to the parent drugs. We found that short-term (48 h) exposure of MCF-7 cells to low (nM) concentrations of doxorubicin or oracin caused a significant elevation of both cytostatics reduction rates. In order to identify principal enzyme(s) catalyzing the reduction of doxorubicin and oracin, we tested effects of selected inhibitors of aldo-keto reductase AKR1C3 and carbonyl reductase CBR1 on doxol and DHO formation. AKR1C3 and CBR1 are expressed in human breast cells. We found that the principal enzyme reducing both drugs was CBR1, while AKR1C3 seems not to play any important role in metabolism of either doxorubicin or oracin. To analyze the expression of AKR1C3 and CBR1 gene in control and drug-treated MCF-7 cells, relative quantification of mRNA using real-time RT-PCR was carried out. The exposure of MCF-7 cells to doxorubicin or oracin led to a non-significant increase of AKR1C3 and CBR1 mRNA levels. We assume that induction of CBR1 is caused by non-transcriptional mechanisms. In conclusion, identification of drug-inactivating enzymes in specific tumor tissue and mechanisms of their induction could contribute to explanation of drug-resistance.