ABSTRACT

Charles University in Prague, Faculty of Pharmacy in Hradec Králové
Department of Biochemical Sciences
Candidate **Mgr. Adam Skarka**
Supervisor **Prof. Ing. Vladimír Wsól, Ph.D.**
Title of Doctoral Thesis **The role of membrane bound enzymes in metabolism of xenobiotics in human**

Heterogeneous substances named xenobiotics are commonly present in wide surroundings of man. Some of them are actively or passively acquired into organism and undergo metabolic transformation, which lead to their excretion. In this case, important role is played by different enzymatic systems. The most known system is superfamily of cytochrome P450s catalyzing oxidation reactions. However, the importance of reducing systems like superfamilies of short chain dehydrogenases/reductases (SDR), middle chain dehydrogenases/reductases (MDR) and aldo keto reductases (AKR) have grown recently. Carbonyl group, the target of reduction catalyzed by SDR, MDR and AKR superfamilies, is also present in various groups of drugs, e.g. antineoplastics and non-steroidal anti-inflammatory drugs. Conversion of this group can lead to the loss of therapeutic effect and/or to the increase of toxicity for organism.

Some xenobiotics contain so called prochiral functional group in their structure, e.g. carbonyl group. Conversion of this group can lead to the formation of chiral products. Ratio of formed products is not random and is unique for each enzyme substrate pair. This ratio is called enzyme stereospecificity. It possesses similar importance as enzymatic kinetics constants and can be significant helper in research of the new enzymes. This property has been demonstrated on many examined substances and enzymes, for example pairs alkyl p tolyl sulfides and FMO, risperidon and CYP450, NNK and CBR1 or AKR1C1, benzimidazole anthelmintics and FMO and phenytoin and CYP450. Comparison of stereospecificity values for whole fractions and separate enzymes can determine whether some other enzyme(s) is/are participating in transformation of chosen substance present in fraction.

Discrepancy between stereospecificity ratios for dihydrooracin (DHO) formation in human liver microsomes (40 % formation of (+)–DHO) and the only liver microsomal carbonyl reductase with described reduction of xenobiotics HSD11B1 (24 % formation of (+)–DHO), suggested presence of another carbonyl reducing enzyme in human liver microsomes. Purification of new carbonyl reductase from human liver tissue was performed. Optimization of whole purification process, which includes solubilization of microsomes and two purification steps, led to acquisition of sufficient amount of unknown carbonyl reductase for its partial characterization. Kinetic constants for reduction of oracin to DHO were comparable with values for cytosolic enzymes. Increase of enzyme purity was proved by increase of specific activity value (activity of enzyme relative to amount of protein). Subsequent detection of enzyme via mass spectrometry was not possible due to very low concentration of protein.

Oracin is potent antitumor drug, whose development was stopped due to economic reasons in the second stage of clinical trials. Very frequently used group of antineoplastics are anthracyclines, like doxorubicin (DOX), daunorubicin (DAUN) and idarubicin (IDA), which are like oracin metabolized by reduction of carbonyl group. Although ANTs are used in long term, detail information about their conversion is missing. On the basis of kinetic constants for reduction of oracin to DHO, involvement of unknown carbonyl reductase on reduction of ANT has been investigated. Only catalysis of DAUN reduction (Km=954±129μM, Vmax=720±27 nmol/min per mg, CLint=0.76 ml/min per mg), which is not very important compared to cytosolic enzymes CBR1, CBR3 and AKRA1A, and catalysis of IDA reduction (Km=301μM, Vmax=158±7 nmol/min per mg, CLint=0.52 ml/min per mg) have been proved. It also had to be checked, what is overall rate of reduction of chosen ANTs in liver microsomes. There has been found that in concentrations higher than 100μM, hydrolysis takes place in and particular aglycones are formed. Kinetic constants for reduction of DOX (Km=248μM, Vmax=0.248±0.025 nmol/min per mg, CLint=0.001 ml/min per mg) were in the same order as

constants known for liver cytosol. These constants couldn’t be counted for reduction of DAUN and IDA.