Abstract

The aim of this Thesis was to evaluate the interaction of optical isomers of chiral drugs with nine of the most important xenobiotic-metabolizing enzymes, cytochromes P450. A key cause of drug-drug interactions is the inhibition of cytochrome P450 enzymes that are responsible for elimination of many drugs. Screening for inhibition potency of P450 by drugs is an important part of drug research.

First part of the work was focused on the ability of chiral drugs to influence enzyme activity of nine most important cytochromes P450 involved in drug metabolism. The inhibition potency of individual enantiomers of one drug was compared. Optical isomers of azole antifungal drugs, known CYP3A4 inhibitors; enantiopure drugs, their excluded enantiomer and racemate; and dihydropyridine calcium channel blockers were studied. Molecular docking was used to verify selected *in vitro* results. Last aim of this work was to evaluate the effect of enantiomers of model drug, amlodipine, on enzyme activity of CYP2C9 and CYP2C19 alleles corresponding to poor, intermediate and extensive activity.

Systematic testing of effect of individual enantiomers on enzyme activities of P450 in human liver microsomes revealed differences in the inhibition potency in most cases. Most interactions were with the most important form, CYP3A4. Its activity was significantly affected by optical isomers of ketoconazole, itraconazole, by enantiomers of tamsulosin, tolterodine, and by all tested dihydropyridine calcium channel blockers. The inhibition potency of enantiomers of individual drugs differed significantly. All significant inhibitions were characterised by mechanism of inhibition and inhibition constant K_i. Relevant inhibition with enantiospecific differences was found also with CYP2C9, CYP2C19 and dihydropyridine calcium channel blockers.

Molecular docking was used to explain the difference in the inhibition between tamsulosin enantiomers on CYP3A enzyme activity. Binding of these compounds was simulated with two typical substrates, testosterone and midazolam. Tamsulosin enantiomers showed different binding poses in the active site of CYP3A4 cavity explaining different inhibition potency towards enzyme activity measured by two different specific probes. Molecular docking was also used to simulate the binding of the enantiomers of amlodipine to the CYP3A4 cavity, where different R- and S-amlodipine binding has again been demonstrated.

As theyinhibit CYP2C9 and CYP2C19, amlodipine enantiomers were used for another experiment, assessing their effect on enzyme activity of CYP2C9 and CYP2C19 with phenotypes corresponding to poor, intermediate, and extensive metabolizer. Results revealed that the inhibition is phenotype independent. However, the enantioselective inhibition by these two enantiomers was confirmed.

The results of this work show the importance of stereoselective disposition for enzyme inhibition. Individual optical isomers of drugs may interact differently with cytochromes P450 and therefore their inhibition potency also differs. Therapeutic effect depends usually on one optical isomer. The clinical use of enantiopure drugs would be beneficial. The required therapeutic effect is retained but it allows lower dosage, fewer adverse effects and lower risk of drug-drug interactions.