Abstract

Ellipticine is an alkaloid with antitumor activity, whose mechanism of action is based on intercalation into DNA, inhibition of topoisomerase II and formation of covalent adducts with DNA, after its enzymatic activation by cytochromes P450 and/or peroxidases. Ellipticine is oxidized by cytochromes P450 to form up to five metabolites (7-hydroxy-, 9-hydroxy, 12-hydroxy-, 13-hydroxyellipticine and N^2-oxide ellipticine). 9-Hydroxy- and 7-hydroxyellipticine are considered to be detoxification metabolites, whereas 12-hydroxy-, 13-hydroxyellipticine and N^2-oxide of ellipticine are considered as activation metabolites, which are responsible for formation of covalent DNA adducts. The aim of this thesis was to examine the efficiency of human recombinant cytochromes P450 expressed in eukaryotic (Supersomes^{TM}) and two prokaryotic expression systems (Bactosomes) in oxidation of ellipticine. Cytochromes P450 expressed in prokaryotic systems differed in the amounts of "coexpressed" NADPH:CYP reductase. The resulting ellipticine metabolites were analyzed by HPLC. The results obtained in this thesis demonstrate that human cytochromes P450 2C9/2D6/2C19 expressed in prokaryotic or eukaryotic systems oxidize ellipticine to form up to four metabolites: 9-hydroxy-, 12-hydroxy-, 13-hydroxyellipticine and N^2-oxide of ellipticine. However, the efficiencies of individual systems differ. CYP2C9/2C19/2D6 expressed in a prokaryotic system with higher levels of NADPH:CYP reductase oxidized ellipticine most effectively, forming preferentially 9-hydroxy-, 12-hydroxy- and 13-hydroxyellipticine. The efficiencies of cytochromes P450 to oxidize ellipticine is as follows: CYP2C19> 2D6 >> 2C9, both in the presence and the absence of cytochrome b_5. On the contrary, the CYP2C9/2C19/2D6 enzymes expressed in a prokaryotic system with lower levels of NADPH:CYP reductase appear to be less effective in ellipticine oxidation. Cytochrome b_5 affects oxidation of ellipticine by CYP2C9, 2C19 and 2D6 expressed in a prokaryotic system with lower levels of NADPH:CYP reductase. The oxidation of ellipticine in this case favored formation of activation metabolites. Furthermore, the effects of added NADPH:CYP reductase on ellipticine oxidation by cytochromes P450 expressed in a prokaryotic system with lower levels of NADPH:CYP reductase were examined.

Keywords: cytochromes P450, ellipticine, NADPH:CYP reduktase, cytochrome b_5, prokaryotic expression system, eukaryotic expression system.