Abstract

Ellipticine is an alkaloid isolated from Apocynaceae plants exhibiting significant antitumor and anti-HIV activities. Cytochromes P450 (CYP) and peroxidases are the enzymes participating in metabolism of ellipticine. This process provides activation and detoxication metabolites of ellipticine. The CYP enzymes, which participate in oxidation of ellipticine in different tissues (liver, lung and kidney) of rat, a model organism simulating the fate of ellipticine in humans have already been identified. In this work, the effects of ellipticine on contents and catalytic activities of CYPs and other components of the mixed-function oxidase (MFO) system in this animal system were studied. For detection of contents of CYPs and other components of the MFO system, spectroscopic and electrochemical methods were used. To determine catalytic activities of CYPs and NADPH:cytochrome P450 reductase, reactions with specific substrates of these enzymes were utilized. The results found in this study demonstrate that expression and catalytic activity of CYP1A is induced by ellipticine in all of the tested organs (liver, kidney and lung) of rats treated with the drug. Moreover in liver, the cytochrome b_5 expression is also induced. In addition, in this organ, expression and catalytic activity of CYP3A was increased by ellipticine. Likewise, the NADPH:cytochrome P450 reductase content and its catalytic activity were changed by ellipticine treatment.

The physiological function of cytochrome P450 2S1 organisms is still unclear. To explain its function and its participation in metabolism of xenobiotics, the pure CYP2S1 is necessary. In order to prepare this CYP, its heterologous expression in the bacterial expression systems of *E. coli* was used. The thesis describes the conditions suitable for production of plasmid pCMV, which contains the *CYP2S1* gene. The membrane fractions of the production bacterial system were isolated. The membrane fraction did contain any component exhibiting absorption maximum at 450 nm, typical for the CYP enzymes. Therefore, the *CYP2S1* gene were modified and inserted into the expression plasmid pBAD-A. This plasmid contains arabinose operon. For production of CYP2S1, other bacterial strains (TOP10 a LMG194) suitable for this regulatory system were used. However, the production of CYP2S1 in these expression systems was not successful again. No CYP2S1 was produced in the membrane fraction of these bacterial strains.