Abstract

An environmental pollutant and a human carcinogen benzo[a]pyrene (BaP) is after its activation with cytochrome P450 (CYP) able to covalently bind to DNA. In the thesis, one of the target was to investigate an influence of individual components of mixed function monooxygenase (MFO) system on metabolism of benzo[a]pyrene and generation of adducts of activated BaP with DNA. The study was particularly focused to increase our knowledge on the effect of cyt b_5 on metabolism of BaP by cytochrome P450 1A1 (CYP1A1) and its potential to serve as a donor of electrons during the reaction cycle of this cytochrome P450. The effect of cyt b_5 on generation of BaP metabolites and adducts of BaP with DNA was investigated. In addition the effect of two different expression systems for cytochrome P450 1A1 (prokaryotic and eukaryotic) was also studied.

The influence of cyt b_5 on oxidation another xenobiotic compound, a plant alkaloid ellipticine that exhibit antitumor activities, was also investigated. Its pharmacological efficiency, as well as side effects depends on its metabolic activation by cytochrome P450. CYP3A4 is very important for ellipticine activation and therefore this enzyme was used in our experiments.

Furthermore, a suitability of rat as a model organism mimicking the metabolic fate of BaP in human was studied.

High performance liquid chromatography (HPLC) was used for separation of metabolites of both compounds. DNA adducts generated by BaP activated by CYPs were analysed by the "^{32}P-postlabeling" method.

The results found in a study investigating the potential of rats to serve as a model organism mimicking a metabolic fate of BaP in humans indicate that human and rat microsomes generate almost the same metabolites, with the only exception of a metabolite BaP-9-ol. This metabolite was not formed by human microsomes. Metabolites generated by rat CYP1A1 differ from metabolites generated by human CYP1A1 also by formation of BaP-4,5-dihydrodiol. This metabolite is not formed by human CYP1A1. Other BaP metabolites generated by rat and human CYP1A1 are identical. This finding indicates that rat is a suitable model mimicking metabolism of BaP in human. Epoxide hydrolase was shown to be the essential enzyme for generation of dihydrodiols of BaP and one of the BaP-DNA adducts. Its lack in a prokaryotic expression system for CYP1A1 (Bactosomes) limited this system to be used for a study of the metabolic fate of BaP and preferred the eukaryotic system expressing CYP1A1 (Supersomes^{TM}). Cyt b_5 stimulates generation of BaP metabolites and BaP-DNA adduct formation by rat and human CYP1A1. The results found in thesis show that the system of human CYP1A1, NADH, NADH:cytochrome b_5 reductase and cytochrome b_5 is able to metabolize BaP and to generate BaP-DNA adducts. They also demonstrate that NADH in this system can act as a sole electron donor both for the first and the second reduction of CYP1A1 during oxidative activation of BaP *in vitro*.

The ability of cyt b_5 donates both electrons to CYP enzyme was also observed in oxidation of ellipticine by human CYP3A4.