## Abstract

Benzo[a]pyrene (BaP) is a human carcinogen, which is metabolized by a variety of enzyms such as cytochrome P450 (CYP) and epoxide hydrolase. The aim of this work was to study BaP metabolism *in vitro* by the hepatic microsomal system of rats treated with CYP inducers and by human cytochrome P450 1A1 (CYP1A1) expressed in eukaryotic and prokaryotic systems. An eukaryotic expression system consisted of microsomes isolated from insect cells, whereas a prokaryotic expression system was formed by the membrane fragments of *E. coli*. In the case of recombinant human CYP1A1, we investigated the influence of cytochrome $b_5$, NADPH:cytochrome P450 reductase (CPR) and epoxide hydrolase in BaP oxidation. Isolation and purification of rabbit hepatic CPR was another aim of this work. BaP metabolites were separated by HPLC. The results found in this work demostrate the fact that hepatic microsomal systems of rats treated with an inducer of CYP1A (Sudan I), an inducer of CYP2B (phenobarbital) and an inducer of CYP3A (PCN) exhibit higher efficiency of BaP oxidation than microsomes of control rats. BaP is oxidized by human CYP1A1 expressed in the eukaryotic system to six metabolites (BaP-9,10-dihydrodiol, BaP metabolite with unknown structure, BaP-7,8-dihydrodiol, BaP-1,6-dion, BaP-3,6-dion, BaP-3-ol), whereas by human CYP1A1 expressed in the prokaryotic system only to four metabolites (BaP metabolite with unknown structure, BaP-1,6-dion, BaP-3,6-dion, BaP-3-ol). Cytochrome $b_5$ has no significant influence on BaP oxidation by human CYP1A1 expressed in both systems. BaP oxidation by human CYP1A1 expressed in the prokaryotic system is influenced by the amount of CPR. The enzymatic system reconstituted from CYP and CPR in a ratio of CYP:CPR of 1:3 (containing the highest amount of CPR) has the highest efficiency in BaP oxidation. Human CYP1A1 expressed in the prokaryotic system reconstituted with CPR in a ratio of CYP:CPR of 1:1 oxidizes BaP to another metabolite, BaP-7.8-dihydrodiol, an intermediate leading to formation of covalent DNA adducts. In the presence of epoxide hydrolase, human CYP1A1 expressed in the prokaryotic system oxidizes BaP to dihydrodiols, namely BaP-9,10-dihydrodiol and BaP-7,8-dihydrodiol. The results obtained in this work contribute to our knowledge of BaP metabolism by human CYP1A1 expressed in model expression systems.



**Key words:**

benzo[a]pyrene, metabolism, cytochrome P450, prokaryotic and eukaryotic expression systems, cytochrome $b_5$, NADPH:cytochrome P450 reductase, epoxid hydrolase, HPLC