ORIGINAL ARTICLE

Modulation of human cytochrome P450 1A1-mediated oxidation of benzo[*a*]pyrene by NADPH:cytochrome P450 oxidoreductase and cytochrome b_5

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Abstract

OBJECTIVES: Cytochrome P450 (CYP) 1A1 located in the membrane of endoplasmic reticulum is the most important enzyme in both activation and detoxification of carcinogenic benzo[*a*]pyrene (BaP), in combination with microsomal epoxide hydrolase (mEH). However, it is still not clearly explained how the electron transfer is mediated by NADPH:CYP oxidoreductase (POR), another component of the microsomal enzymatic system, on CYP1A1 during BaP oxidation, and whether microsomal cytochrome b_5 might influence this electron transfer. **METHODS:** High performance liquid chromatography (HPLC) was employed for separation of BaP metabolites formed by enzymatic systems containing human CYP1A1. **RESULTS:** Human CYP1A1 expressed with POR in eukaryotic and prokaryotic expression cellular systems, in microsomes of insect cells (Supersomes™) and in a membrane fraction of *Escherichia coli*, respectively, and these enzyme systems reconstituted with purified cytochrome b_5 were utilized to study BaP oxidation. Human CYP1A1 expressed in Supersomes™ oxidized BaP to seven metabolites [7,8- and 9,10-dihydrodiols, 1,6-dione, 3,6-dione, 3- and 9-phenols, and a metabolite with unknown structure (Mx)], whereas this enzyme expressed in membranes of *E. coli* formed only the metabolites 1,6- and 3,6-diones, 3- and 9-phenols, and Mx. Addition of cytochrome b_5 to CYP1A1 expressed in the eukaryotic system led to a more than 2-fold increase in BaP metabolism, but had essentially no effect on BaP oxidation by CYP1A1 expressed in *E. coli*. **CONCLUSION:** The effect of cytochrome b_5 on CYP1A1 conformation and the electron transfer to this enzyme may contribute to the cytochrome b_5-mediated stimulation of BaP oxidation.

Abbreviations:

BaP	- benzo[a]pyrene
BPDE	- BaP-7,8-dihydrodiol-9,10-epoxide
CYP	- cytochrome P450
dG-N^2-BPDE	- 10-(deoxyguanosin-N^2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo-[a]pyrene
DLPC	- 1,2-dilauroylphosphatidylcholine
DMSO	- dimethyl sulfoxide
HPLC	- high performance liquid chromatography
HRN	- Hepatic P450 Reductase Null
mEH	- microsomal epoxide hydrolase
NADPH	- nicotinamidadeninedinucleotide phosphate (reduced)
NMR	- nuclear magnetic resonance
PAH	- polycyclic aromatic hydrocarbon
POR	- NADPH:cytochrome P450 oxidoreductase
RCN	- Reductase Conditional Null
r. t.	- retention time
UV	- ultraviolet
WT	- wild-type

INTRODUCTION

Benzo[*a*]pyrene (BaP) is a polycyclic aromatic hydrocarbon (PAH) that has been classified as human carcinogen (Group 1) by the International Agency for Research on Cancer (IARC, 2010). BaP requires metabolic activation catalyzed by cytochrome P450 (CYP) enzymes prior to reaction with DNA (Baird *et al.* 2005). Of the CYP enzymes, CYP1A1 is one of the most important CYP enzymes in metabolic activation of BaP to species forming DNA adducts (Baird *et al.* 2005; Hamouchene *et al.* 2011), in combination with microsomal epoxide hydrolase (mEH). First, CYP1A1 oxidizes BaP to an epoxide that is then converted to a dihydrodiol by mEH (*i.e.* BaP-7,8-dihydrodiol); then further bioactivation by CYP1A1 leads to the ultimately reactive species, BaP-7,8-dihydrodiol-9,10-epoxide (BPDE) that can react with DNA, forming adducts preferentially at guanine residues. The 10-(deoxyguanosin-N^2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (dG-N^2-BPDE) adduct is the major product of the reaction of BPDE with DNA *in vitro* and *in vivo* (Bauer *et al.* 1995; Arlt *et al.* 2008; 2012).

BaP is, however, oxidized also to other metabolites such as other dihydrodiols, BaP-diones and hydroxylated metabolites (Bauer *et al.* 1995; Chun *et al.* 1996; Kim *et al.* 1998; Baird *et al.* 2005; Jiang *et al.* 2007; Zhu *et al.* 2008). Even though most of these metabolites are detoxification products, BaP-9-ol is a precursor of 9-hydroxy-BaP-4,5-epoxide that can form another adduct with deoxyguanosine in DNA (Schoket *et al.* 1989; Nesnow *et al.* 1993; Fang *et al.* 2001; Stiborova *et al.* 2014). Therefore, regulation of CYP1A1-mediated oxidation of BaP leading to either metabolites forming BPDE, 9-hydroxy-BaP-4,5-epoxide or the BaP metabolites that are the detoxification products is of major importance.

Beside CYP1A1, CYP1B1 also oxidizes BaP, forming both the detoxification and activation metabolites. Its efficiency is however about half of that of CYP1A1. Among other CYP enzymes, CYP1A2, 2C8/9/19, 2E1, and 3A4 also oxidize BaP, but their efficiencies are more one order of magnitude lower than those of CYP1A1 (Bauer *et al.* 1995; Kim *et al.* 1998; Baird *et al.* 2005;).

CYP enzymes, including CYP1A1, are a component of a mixed function oxidase system located in the membrane of endoplasmic reticulum that contains beside the CYPs also another enzyme, NADPH:cytochrome P450 oxidoreductase (POR), and cytochrome b_5 accompanied with its NADH:cytochrome b_5 reductase. *Via* the activation of molecular oxygen, this multienzyme system catalyzes the monooxygenation of a variety of xenobiotics, including BaP (Coon, 1978). The oxygen is activated in the active center of CYPs by two electrons transferred from NADPH and/or NADH by means of POR and/or cytochrome b_5, respectively. Whereas POR is an essential constituent of the electron transport chain towards CYP, the role of cytochrome b_5 is still quite enigmatic (Porter, 2002; Schenkman and Jansson, 2003; Finn *et al.* 2008; McLaughin *et al.* 2010; Kotrbova *et al.* 2011; Stiborova *et al.* 2012; Sulc *et al.* 2012; Hendersson *et al.* 2013; Indra *et al.* 2013). In fact, for CYP1A1 the influence of the POR-mediated electron transfer from NADPH to this CYP by cytochrome b_5 is essentially not known. Moreover, a role of POR in the oxidative metabolism of BaP is not clearly established. Recently we found that in two mouse models [i.e. Hepatic P450 Reductase Null (HRN) and Reductase Conditional Null (RCN)], in which the expression of POR has been permanently or conditionally deleted in liver leading to a lack of almost all POR activity, the levels of the CYP- and mEH-mediated dG-N^2-BPDE adducts in livers of HRN and RCN mice treated with BaP were higher than in BaP-treated wild-type (WT) mice (Arlt *et al.* 2008; 2012; Stiborova *et al.* 2014). Therefore, in the present study we investigated the effect of POR and cytochrome b_5 on a potency of CYP1A1 to oxidize BaP.

Several model systems containing human CYPs, such as human hepatic microsomes, cells in culture including human hepatocytes, purified CYP enzymes reconstituted with POR in liposomes and/or human CYP enzymes overexpressed in baculovirus/insect cell, yeast, *Salmonella*, and other cellular systems, have already been utilized to study metabolism of several xenobiotics including BaP *in vitro* (Guengerich and Parikh, 1997; Anzenbacher and Anzenbacherova, 2001; Schwarz *et al.* 2001; Shimada *et al.* 2001; 2004; Zuber *et al.* 2002; Kramer and Tracy, 2008; Guguen-Guillouzo and Guillouzo, 2010; Davydov, 2011). However, which CYPs are most suitable for such metabolic studies remain to be further examined. In this work, human recombinant CYP1A1 expressed with its reductase (POR) in microsomes isolated from insect cells transfected with baculovirus constructs containing cDNA of human CYP1A1 and POR (Supersomes™) and in a membrane fraction of *Escherichia coli* cells transfected with *cDNA* of human CYP1A1 and/or these systems reconstituted with purified POR and/or cytochrome b_5 were used as model

systems. In addition, human hepatic microsomes containing a natural spectrum of human CYPs and other enzymes located in a membrane of endoplasmic reticulum were used as a positive control.

MATERIAL AND METHODS

Microsomal and enzymatic incubations

Male human hepatic microsomes (pooled sample; cat. no. 452172) and Supersomes™, microsomes isolated from insect cells transfected with a baculovirus construct containing cDNA of human CYP1A1 and expressing POR (CYP1A1 expressed in a eukaryotic system), were purchased from Gentest Corp. (Woburn, MI, USA) and used to study of BaP (Sigma Chemical Co, St Louis, MO, USA) oxidation. Bactosomes, a membrane fraction isolated from cells of *E. coli* transfected with construct of cDNA of human CYP1A1 and expressing either low (i.e. CYP1A1LR) or high levels of POR (i.e. CYP1A1R) were obtained from Cypex (BioDundee, Dundee, UK). Incubation mixtures used for studying BaP metabolism in human hepatic microsomes or in Supersomes™ and Bactosomes contained 100 mM potassium phosphate buffer (pH 7.4), NADPH-generating system [1 mM $NADP^+$, 10 mM D-glucose-6-phosphate, 1 U/ml D-glucose-6-phosphate dehydrogenase (all from Sigma Chemical)], 0.5 mg of microsomal protein or 100 nM CYP1A1 in Supersomes™ or Bactosomes, 50 µM BaP (dissolved in 5 µl dimethyl sulfoxide) in a final volume of 500 µl. In several experiments, these CYP1A1 systems were reconstituted with POR (CYP1A1LR), with cytochrome b_5 (all CYP1A1 systems) and/or mEH (CYP-1A1LR). The enzyme reconstitution utilizing the above systems (Supersomes™ and Bactosomes) and pure POR, cytochrome b_5 and/or mEH or POR in liposomes prepared from phospholipids such as 1,2-dilauroylphosphatidylcholine (DLPC) (Sigma) was performed as described (Stiborova *et al.* 2002; 2005; 2006; 2012; 2014; Dracinska *et al.* 2006; Kotrbova *et al.* 2011), using different molar ratios of CYP1A1 to POR and cytochrome b_5 (see Results for details). The reaction was initiated by adding 50 µl of the NADPH-generating system. Control incubations were carried out either without enzymatic system (microsomes or the CYP1A1 systems), or without NADPH-generating system, or without BaP. After incubation (37 °C, 20 min), 5 µl of 1 mM phenacetin (Sigma) in methanol was added as an internal standard. BaP metabolites were extracted twice with ethyl acetate (2 × 1 ml), solvent evaporated to dryness, residues dissolved in 25 µl methanol and BaP metabolites separated by high performance liquid chromatography (HPLC).

Isolation of POR, cytochrome b_5 and mEH

Rabbit liver POR was purified as described (Stiborova *et al.* 2002). Cytochrome b_5 was isolated from rabbit liver microsomes by the procedure described by Roos (1996). mEH was purified from liver microsomes of rabbits pretreated with phenobarbital as described by Ariyoshi *et al.* (1994). These enzymes were used for the reconstitution experiments.

HPLC analysis of BaP metabolites

HPLC analysis of BaP metabolites was performed on a Nucleosil® C18 reverse phase column, (250 × 4 mm, 5 µm; Macherey Nagel, Düren, Germany) using a Dionex system consisting of a pump P580, a UV/VIS Detector UVD 170S/340S, an ASI-100 Automated Sample Injector, a termobox COLUMN OVEN LCO 101 and an In-Line Mobile Phase Degasser Degasys DG-1210 Dionex controlled with Chromeleon™ 6.11 build 490 software. The conditions used for the chromatographic separation of BaP metabolites were as follows: 50% acetonitrile in water (*v/v*) with a linear gradient to 85% acetonitrile in 35 min, then an isocratic elution with 85% acetonitrile for 5 min, a linear gradient from 85% acetonitrile to 50% acetonitrile in 5 min, followed by an isocratic elution of 50% acetonitrile for 5 min (Moserova *et al.* 2009). Detection was by UV at 254 nm. Recoveries of BaP metabolites were around 95%. BaP metabolite peaks (Figure 1) were collected and analyzed by NMR and/or mass spectrometry as described recently (Indra *et al.* 2013; Stiborova *et al.* 2014). The peak areas at 254 nm were calculated relative to the peak area of the internal standard phenacetin, and expressed as relative peak areas.

Statistical analyses

For statistical data analysis we used Student's *t*-test. All *p*-values are two-tailed and considered significant at the 0.05 level.

RESULTS

Oxidation of BaP by human hepatic microsomes

Human hepatic microsomes are a natural system containing all components of a monooxygenase system located in a membrane of endoplasmic reticulum, CYPs, POR, cytochrome b_5 and its reductase, NADH: cytochrome b_5, reductase, in addition to mEH. Human hepatic microsomes oxidized BaP to six metabolites that were separated by HPLC (Figure 1A). The metabolites formed from BaP by human hepatic microsomes were identified by NMR and/or mass spectrometry (Indra *et al.* 2013; Stiborova *et al.* 2014) to be BaP-9,10-dihydrodiol (M1), BaP-4,5-dihydrodiol (M2), BaP-7,8-dihydrodiol (M3), BaP-1,6-dione (M4), BaP-3,6-dione (M5), and BaP-3-ol (M7), all corresponding to the metabolites that were formed by CYP1A1 in combination with mEH in other studies (Bauer *et al.* 1995; Kim *et al.* 1998; Baird *et al.* 2005; Moserova *et al.* 2009; Stiborova *et al.* 2014) (see Figures 1A and 2). Essentially no BaP metabolites were found when NADPH, a cofactor of the POR-dependent CYP monooxygenase system, was not present in the incubation mixtures (data not shown).

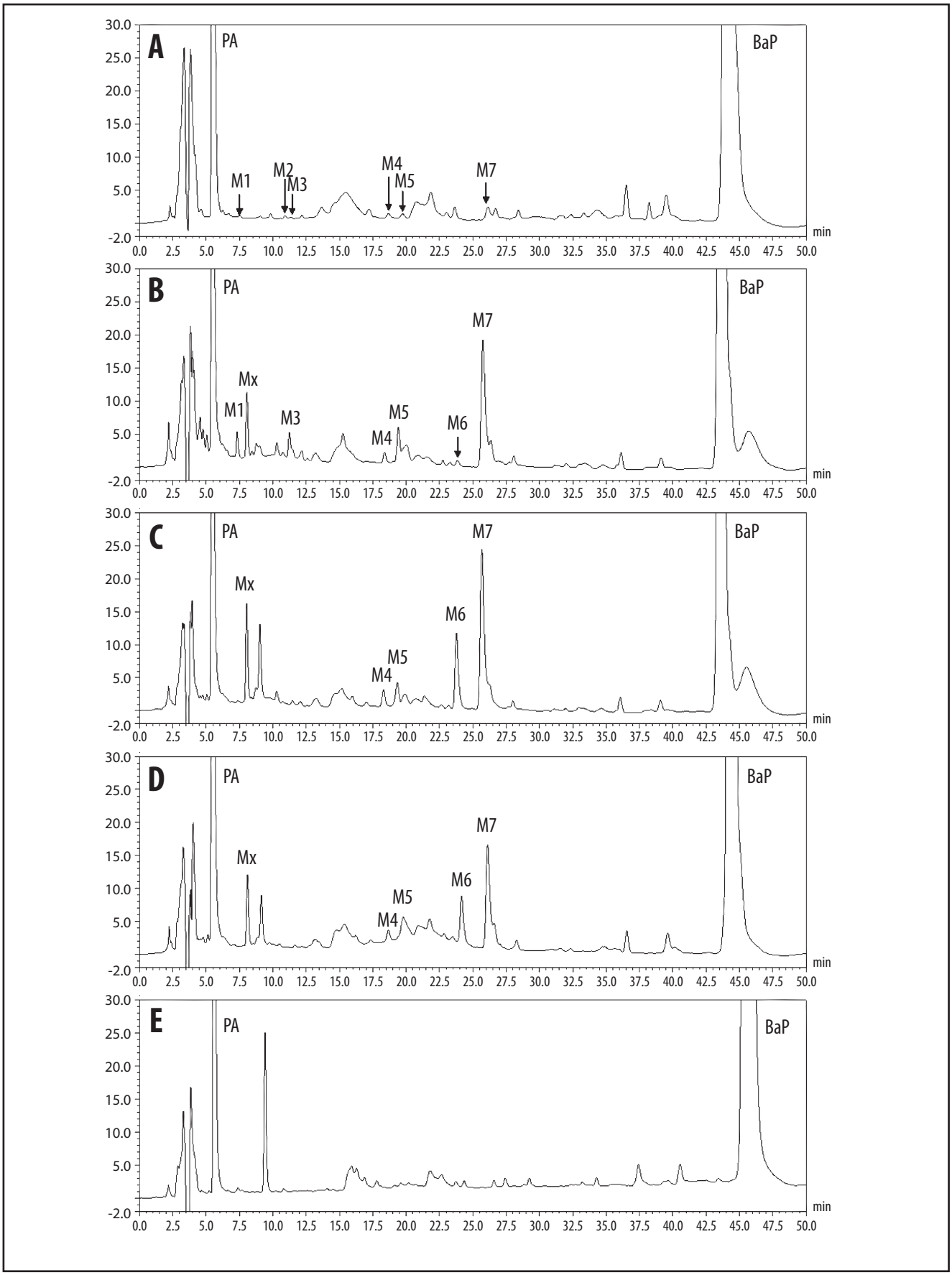

Fig. 1. HPLC of BaP metabolites formed by human hepatic microsomes (**A**), human recombinant CYP1A1 expressed in Supersomes™ (**B**), human recombinant CYP1A1 expressed in a membrane of *E. coli* - CYP1A1R (**C**) and CYP1A1LR (**D**). (**E**) HPLC of control incubation mixture containing BaP and CYP1A1LR, but without the NADPH-generating system. For BaP metabolites M1-M7 and Mx, see Fig. 2. PA, phenacetin.

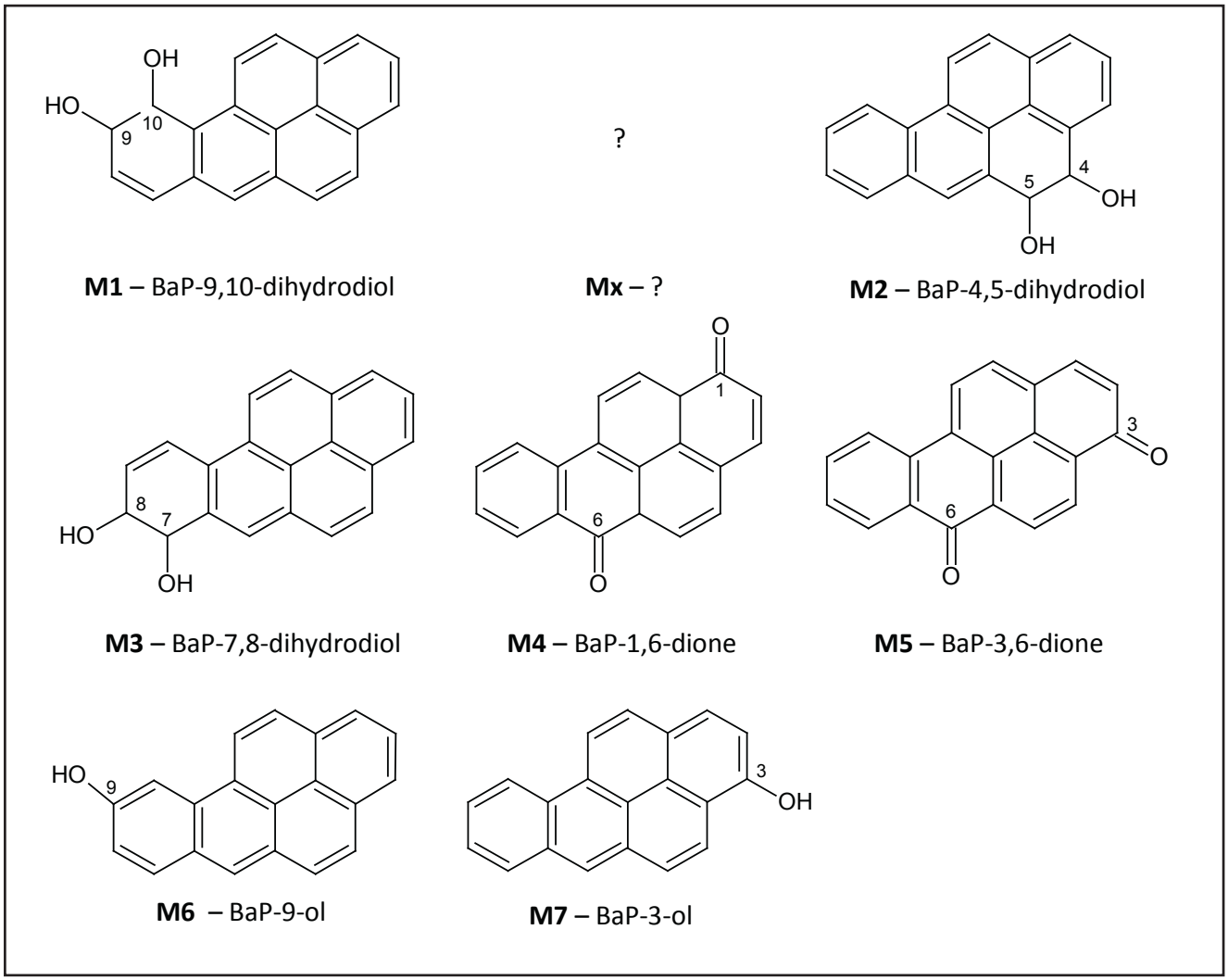

Fig. 2. BaP metabolites formed by human hepatic microsomes and human recombinant CYP1A1

Oxidation of BaP by human CYP1A1 expressed in a eukaryotic cellular system (Supersomes™)

Human CYP1A1 expressed with POR in a microsomal system of Supersomes™ oxidized BaP to seven metabolites, namely BaP-9,10-dihydrodiol, a metabolite assigned as Mx, whose structure has not been identified as yet, BaP-7,8-dihydrodiol, BaP-1,6-dione, BaP-3,6-dione, BaP-9-ol and BaP-3-ol (Figures 1B and 3). One of the dihydrodiols formed by human hepatic microsomes, BaP-4,5-dihydrodiol (M2), has not been detected in this CYP1A1 system. The highest amount of BaP-3-ol was generated by CYP1A1 in Supersomes™ (Figure 1B). The results found using this human CYP1A1 system indicated that BaP is metabolized not only by CYP1A1 present in this enzyme system, but also by mEH, which is important for the hydration of BaP epoxides to produce dihydrodiols. Therefore, this enzyme was expressed in microsomes of the Supersomal system. Essentially no BaP metabolites were found when the NADPH-generating system was deleted from the incubation mixtures (data not shown).

Addition of cytochrome b_5, to CYP1A1 in Supersomes™ led to up to a more than 2-fold increase in BaP oxidation to its metabolites. The highest stimulation effect of cytochrome b_5 has been found on formation of BaP-3-ol and BaP-7,8-dihydrodiol, followed by the effect on generation of BaP-9,10-dihydrodiol, BaP-1,6-dione, BaP-9-ol, a metabolite Mx, and BaP-3,6-dione (Figure 3).

Oxidation of BaP by human CYP1A1 expressed in a prokaryotic cellular system of E. coli (Bactosomes)

Two types of human CYP1A1 enzymatic systems expressed in prokaryotic cells were used to analyze oxidation of BaP. Namely, Bactosomes, a membrane fraction isolated from cells of *E. coli*, containing human CYP1A1 and expressing low or high levels of POR, CYP1A1LR (using a CYP1A1:POR ratio of 1:0.4) or CYP1A1R (using a CYP1A1:POR ratio of 1:0.8), respectively, were employed. Human CYP1A1 expressed in both two *E. coli* systems oxidized BaP to five metabolites; BaP-1,6-dione, BaP-3,6-dione, BaP-9-ol, BaP-3-ol

and a metabolite Mx, whereas no BaP-dihydrodiols were detected (Figure 1C and 1D). Essentially no BaP metabolites were found when the NADPH-generating system was deleted from the incubation mixture containing BaP and the CYP1A1LR (Figure 1E) or CYP1A1R (data not shown) systems.

Of the BaP metabolites formed, BaP-3-ol, BaP-9-ol and a metabolite Mx were the major BaP metabolites, whereas BaP-1,6-dione and BaP-3,6-dione were generated in much lower amounts (Figures 1 and 3). BaP-9-ol metabolite was formed in more than the 3.5-fold higher amounts in this CYP1A1 system than by CYP1A1 in Supersomes™ (Figures 1 and 3). The results found in experiments using a membrane fraction of *E. coli* containing CYP1A1 and POR indicated that mEH, which is the enzyme important for the hydration of BaP epoxides to dihydrodiols, seems to be present in very low concentrations that are not sufficient for catalysis of these reactions.

As shown in Figure 3, only an up to 1.6-fold higher efficiency of the CYP1A1R *E. coli* system containing higher expression levels of POR to oxidize BaP than CYP1A1LR was found. This finding indicates that even the low amounts of POR are capable of an efficient transfer of electrons from the POR cofactor, NADPH, to CYP1A1 during BaP oxidation in this enzymatic system. In order to investigate the effect of various amounts of POR on the electron transport from NADPH to CYP1A1 in BaP oxidation in more detail, CYP1A1LR was reconstituted with increasing concentrations of POR and used as an additional enzymatic system for testing BaP oxidation.

No significant changes in amounts of most BaP metabolites and their profiles were produced by the addition of POR to the CYP1A1LR system until its concentration was equimolar to CYP1A1. Only BaP-3-ol was increased significantly under these conditions. However, under CYP1A1 to POR ratios of 1:2 or 1:3, a significant increase in BaP oxidation was caused by CYP1A1, mainly to the detoxification metabolite BaP-3-ol (Figure 4). Moreover, the low but detectable amounts of BaP-4,5-dihydrodiol and BaP-7,8-dihydrodiol were also produced under these CYP1A1 to POR ratios, indicating that low levels of mEH are expressed in the membrane of *E. coli* (and under the conditions suitable for effective oxidation of BaP to its metabolites) are capable of catalyzing the hydration of BaP-4,5-epoxide and BaP-7,8-epoxide. Similar changes in BaP metabolite profiles were also found in experiments in which POR was added in its liposomal form [POR introduced into liposomes simulating the membrane of endoplasmic reticulum (microsomes)], prepared from membrane phospholipids such as DLPC (Stiborova *et al.* 2002; 2006; 2012, Kotrbova *et al.* 2011) (data not shown). All these findings suggested that the membrane of *E. coli* provides a suitable environment for the appropriate conformation of POR and CYP1A1 proteins to form a reconstituted system catalyzing BaP oxidation.

In contrast to the stimulation effect of cytochrome b_5 on BaP oxidation by human CYP1A1 in Supersomes™, essentially no such effect was detected in the system of CYP1A1 expressed in *E. coli*. Only production of BaP-3-ol by the system with low expression of POR (CYP1A1LR) was significantly increased by addition of

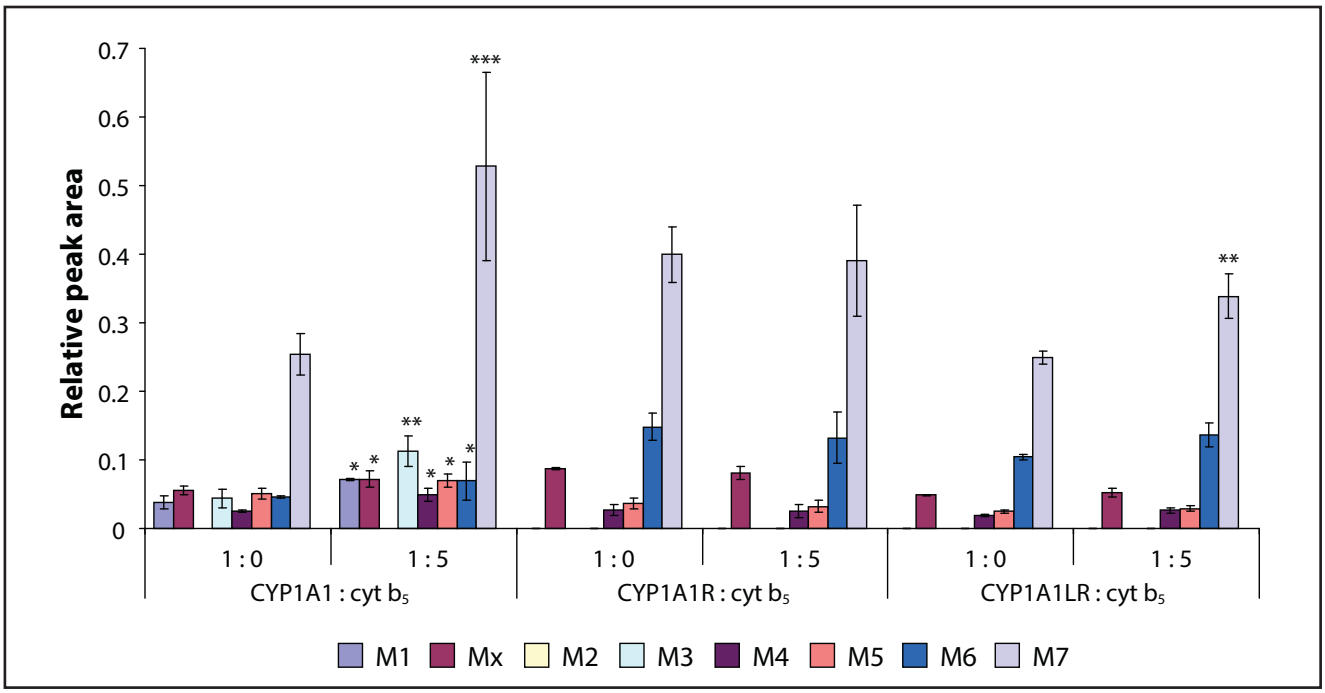

Fig. 3. Oxidation of BaP by human recombinant CYP1A1 expressed in Supersomes™ (CYP1A1) and in a membrane of *E. coli* (CYP1A1R and CYP1A1LR) and the effect of cytochrome b_5 on this oxidation. Comparison was performed by *t*-test analysis; *$p<0.05$, **$p<0.01$, ***$p<0.001$, different from CYP1A1-mediated oxidation of BaP without cytochrome b_5. For BaP metabolites M1-M7 and Mx, see Fig. 2.

cytochrome b_5 (Figure 3). The reason for the opposite effects of cytochrome b_5 on CYP1A1 expressed either in eukaryotic or prokaryotic systems is not yet known. Nevertheless, one can speculate that cytochrome b_5 in the membrane of *E. coli* is not in a conformation appropriate for its function in the CYP1A1 system.

DISCUSSION

The results of this work showed that oxidation of BaP by human CYP1A1 is dependent on a variety of factors. Our experiments utilizing different enzymatic systems of human CYP1A1 showed that one of the most important factors determining efficiency of BaP metabolism is, beside expression of individual enzymes of the microsomal system (CYP1A1, POR, cytochrome b_5 and mEH), mainly the ratios among these enzymes. Other factors determining the efficiency of BaP metabolism are the properties of the subcellular (microsomes, a membrane of *E.coli*) or artificial system (liposomes), i.e. how they simulate the membrane of endoplasmic reticulum. Our results demonstrated that the system of human CYP1A1 expressed in microsomes of eukaryotic cells (Supersomes™) is the better suited enzymatic system for the investigation of BaP metabolism than CYP1A1 expressed in prokaryotic cells of *E. coli*. The low (if any) mEH expression levels, which are essential for the formation of BaP-dihydrodiols, makes this system insufficient for formation of the whole spectrum of BaP metabolites generated during the first (derivative) phase of BaP biotransformation. Nevertheless, the bacterial expression system of *E. coli* was appropriate to evaluate the effect of different concentrations of POR in the CYP1A1 enzymatic system on BaP oxidation. Interestingly, even under low concentrations of POR (using a ratio of CYP1A1 to POR of 1:0.4), CYP1A1 was capable of oxidizing BaP, and increased POR levels in the CYP1A1 reconstituted system resulted in an increase of the BaP detoxification metabolite BaP-3-ol. These results might, to some extent, explain our findings in experiments utilizing the HRN and RCN mouse models indicating that hepatic CYP enzymes in these mouse models seem to be more important for detoxification of BaP *in vivo* despite being important for its bioactivation to form BaP-DNA adducts *in vitro* (Arlt *et al.* 2008; 2012).

The results of this study also showed that the addition of cytochrome b_5 to human CYP1A1 in Supersomes™ resulted in an increased formation of BaP metabolites. Our *in vitro* experiments in the present study, together with previous findings showing that BaP acts as an inducer of cytochrome b_5 (Arlt *et al.* 2012) indicate the potential importance of this protein to greatly influence BaP oxidation *in vivo*. Interestingly, in the case of human CYP1A1 expressed in the membrane of prokaryotic cells of *E. coli*, addition of cytochrome b_5 led to almost no stimulation of BaP oxidation; only oxidation of BaP to BaP-3-ol by the system with low expression of POR was enhanced by cytochrome b_5.

A stimulation of CYP1A1-mediated catalysis by cytochrome b_5 has already been found in the oxidation of its marker substrate Sudan I (Stiborova *et al.* 1988; 2005; 2006) and an anticancer drug ellipticine (Kotr-

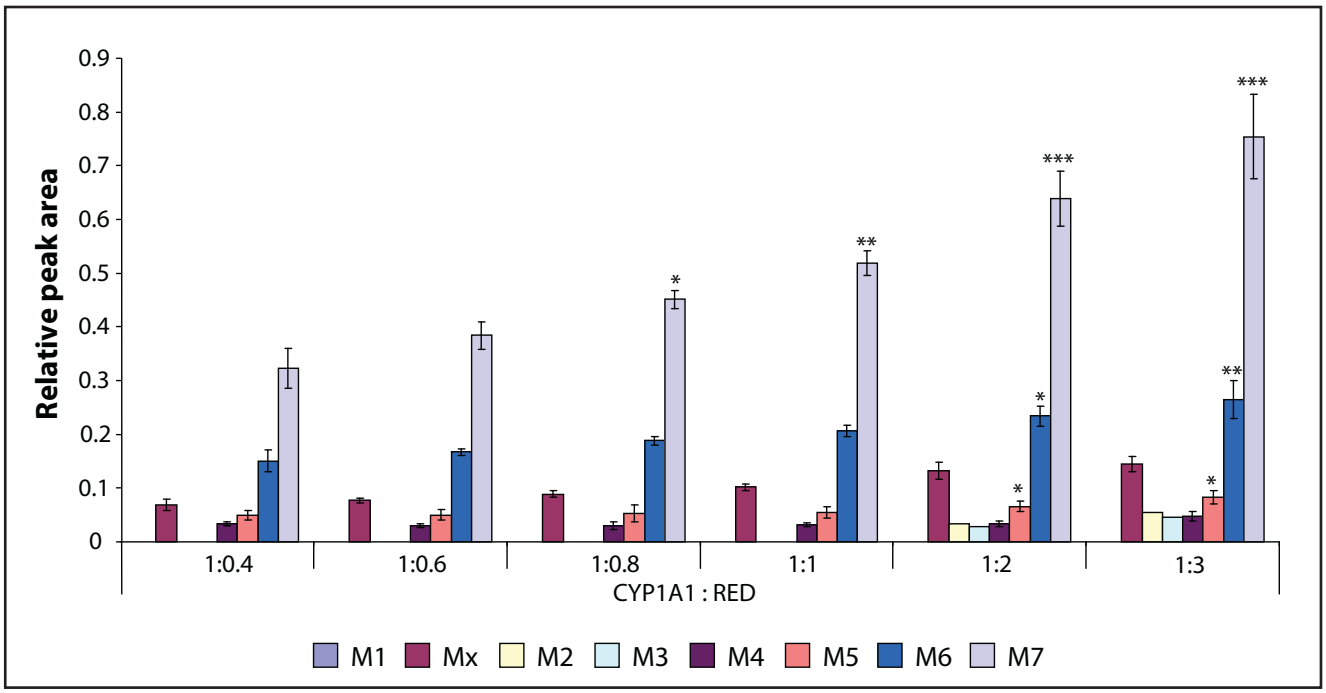

Fig. 4. The effect of a ratio of CYP1A1 to POR on oxidation of BaP by human CYP1A1 expressed in a membrane of *E. coli*. Comparison was performed by *t*-test analysis; *p<0.05, **p<0.01, ***p<0.001, different from oxidation of BaP by CYP1A1LR without addition of POR. For BaP metabolites M1-M7 and Mx, see Fig. 2.

bova *et al.* 2011), but not in that of 7-ethoxyresorufin (Stiborova *et al.* 2005). Two mechanisms of cytochrome b_5-mediated modulation of CYP catalysis have been suggested previously: it can affect the CYP catalytic activities by donating the second electron to CYP in a CYP catalytic cycle and/or act as an allosteric modifier of the oxygenase (Yamazaki *et al.* 1997; 2001; Loughran *et al.* 2001; Porter, 2002; Zhang *et al.* 2005; Schenkman and Jansson, 2003; Guengerich, 2005; Kotrbova *et al.* 2009; 2011; Stiborova *et al.* 2012). The mechanism(s) underlying such allosteric effects, based on reports that apo-cytochrome b_5 can stimulate CYP catalysis, remains uncertain. However, it does seem clear that cytochrome b_5 binding can cause conformational changes to the substrate access channel and binding pocket in the CYP enzyme (Yamazaki *et al.* 1997; 2003; Loughran *et al.* 2001; Porter, 2002; Zhang *et al.* 2005; Schenkman and Jansson, 2003; Guengerich, 2005; Kotrbova *et al.* 2009; 2011; Stiborova *et al.* 2012; Estrada *et al.* 2014). Addition of cytochrome b_5 changed the levels of individual BaP metabolites formed by CYP1A1, and, partially their profiles. Thus interaction of CYP1A1 with cytochrome b_5 can result both in conformational change of the CYP1A1 protein molecule as well as impact on the electron transfer from cytochrome b_5 to CYP1A1, thereby providing mechanisms explaining the observed increase in BaP oxidation. Nevertheless, the real mechanism responsible for the effects of cytochrome b_5 on CYP1A1-mediated oxidation of BaP and a variety of other substrates (e.g. ellipticine) (Kotrbova *et al.* 2011) needs to be explored in further investigations.

CONCLUSION

The results found in this study indicated that the POR-mediated electron transfer from NADPH to human CYP1A1, which is one of the key steps in oxidation of carcinogenic BaP, is mediated by even low concentrations of POR using a ratio of CYP1A1 to POR equaling to 1:0.4. Moreover, BaP oxidation by human CYP1A1 expressed in microsomes of eukaryotic (insect) cells was stimulated by the heme protein cytochrome b_5 that finally leads to more effective oxidative metabolism of this carcinogen. Because of this effect, our study suggests that cytochrome b_5 is an important biological factor influencing BaP-mediated carcinogenesis.

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