ORIGINAL ARTICLE

Oxidation of 3-aminobenzanthrone, a human metabolite of carcinogenic environmental pollutant 3-nitrobenzanthrone, by cytochromes P450 – similarity between human and rat enzymes

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Abstract

OBJECTIVES: 3-Aminobenzanthrone (3-ABA) is the main human metabolite of carcinogenic environmental pollutant 3-nitrobenzanthrone (3-NBA). Understanding which cytochrome P450 (CYP) enzymes are involved in metabolism of this toxicant is important in the assessment of individual susceptibility. Characterization of 3-ABA metabolites formed by rat hepatic microsomes containing cytochromes P450 (CYPs) and identification of the major rat and human CYPs participating in this process are aims of this study.

METHODS: HPLC with UV detection was employed for the separation and characterization of 3-ABA metabolites. Inducers and inhibitors of CYPs and rat and human recombinant CYPs were used to characterize the enzymes participating in 3-ABA oxidation.

RESULTS: Selective CYP inhibitors and hepatic microsomes of rats pre-treated with specific CYP inducers were used to characterize rat liver CYPs metabolizing 3-ABA (measured as consumption of 3-ABA). Kinetics of these reactions catalyzed by rat hepatic microsomes was also evaluated. Based on these studies, we attribute most of 3-ABA metabolism in rat liver to CYP1A and 3A. Among recombinant rat and human CYP enzymes tested in this study, rat CYP3A2 and human CYP3A4/5, followed by CYP1A1 of both organisms were the most effective enzymes converting 3-ABA. Rat hepatic CYP enzymes oxidize 3-ABA up to three metabolites. Two of them were identified to be the products formed by oxidation of 3-ABA on its amino group back to the parent compound from which 3-ABA is generated in organisms, 3-NBA. Namely, *N*-hydroxylation metabolite, *N*-hydroxy-3-ABA and 3-NBA were identified to be these 3-ABA oxidation products. These metabolites are formed by CYPs of a 1A subfamily. Another 3-ABA metabolite, whose structure remains to be characterized, is generated not only by CYP1A but also by other CYP enzymes, predominantly by CYPs of a 3A subfamily.

CONCLUSION: The results found in this study, the first report on the metabolism of 3-ABA by human and rat CYPs, clearly demonstrate that CYPs of 3A and 1A subfamilies are the major enzymes metabolizing 3-ABA.

Abbreviations & units

3-ABA	– 3-aminobenzanthrone
α-NF	– α-naphthoflavone
β-NF	– naphthoflavone
cDNA	– complementary DNA
CYP	– cytochrome P450
DDTC	– diethyldithiocarbamic acid
HPLC	– high performance liquid chromatography
K_m	– Michaelis constant
MS	– microsomes
N-hydroxy-3-ABA	– *N*-hydroxy-3- aminobenzanthrone
NADPH	– nicotinamidadeninedinucleotide phosphate (reduced)
3-NBA	– 3-nitrobenzatntrone
PB	– phenobarbital
RP	reverse phase
r.t.	– retention time
UV	– ultraviolet
VIS	– visible
V_{max}	– maximum reaction rate

INTRODUCTION

The nitroaromatic compound 3-nitrobenzanthrone (3-nitro-7H-benz[de]anthracen-7-one, 3-NBA, Fig. 1) is one of the most potent mutagens and a suspected human carcinogen that is found in diesel exhaust and ambient air pollution (Arlt, 2005; Hansen *et al.* 2007). We found that 3-NBA is activated to *N*-hydroxy-3-aminobenzanthrone (N-hydroxy-3-ABA) by cytosolic and microsomal reductases by simple nitroreduction (Arlt, 2005; Arlt *et al.* 2002, 2003a,b,c, 2005; Stiborova *et al.* 2006a, 2008, 2009; Svobodova *et al.* 2007) (***Figure 1***).

Recently 3-NBA has received much attention due to its extremely high mutagenic potency in the Ames Salmonella assay (Enya *et al.* 1997; Seidel *et al.* 2002; Arlt, 2005). 3-NBA is carcinogenic in rats, causing lung tumours after intratracheal instillation, and it is also a suspected human carcinogen (Seidel *et al.* 2002; Arlt, 2005; Nagy *et al.* 2005).

The uptake of 3-NBA in humans has been demonstrated by the detection of its metabolite 3-aminobenzanthrone (3-ABA, Figure 1) in urine samples of salt mine workers occupationally exposed to diesel emissions (Seidel *et al.* 2002). 3-ABA was also the main metabolite of 3-NBA formed in human fetal bronchial cells and rat lung alveolar type II cells (Borlak *et al.* 2000).

3-ABA was also evaluated to be suitable for coloration of microporous polyethylene films, which are widely used for practical purposes such as separation of liquid mixtures, in particular, as separation membranes in chemical batteries (Grabchev *et al.* 2002), or an advantageous fluorescent phospholipid membrane label in the form of its *N*-palmitoyl derivative (Sykora *et al.* 2002). This suggests industrial and/or laboratory utilization of this 3-NBA metabolite, leading to a putative exposure of people. This is a matter of concern, because we have demonstrated the genotoxicity of both 3-NBA and 3-ABA by the detection of specific DNA adducts formed *in vitro* and *in vivo* (Arlt *et al.* 2001, 2002, 2003a, b, c, 2004a, c; 2005, 2006b; Bieler *et al.* 1999, 2005, 2007; Stiborova *et al.* 2006a, 2008, 2009). Previous work indicated that *N*-hydroxy-3-ABA appears to be the critical intermediate in 3-NBA/ABA-derived DNA adduct formation (Figure 1), which can be further activated by *N,O*-acetyltransferases (NATs) and sulfotransferases (SULTs) (Arlt *et al.* 2002, 2003a, b, 2005). The predominant DNA adducts formed from 3-NBA and 3-ABA are 2-(2'-deoxyguanosin-N^2-yl)-3-aminobenzanthrone (dG-N^2-ABA) and *N*-(2'-deoxyguanosin-8-yl)-3-aminobenzanthrone (dG-C8-*N*-ABA) and these are most probably responsible for the induction of GC to TA transversion mutations induced by these toxicants (Arlt *et al.* 2004a; Arlt *et al.* 2006b; Bieler *et al.* 2007).

Understanding which enzymes are involved in the metabolism (activation and/or detoxication) of 3-ABA is important in the assessment of susceptibility to this 3-NBA metabolite. Recently, we have found that cytochromes P450 (CYP) 1A1 and 1A2 are essential for 3-ABA oxidative activation in human and rat liver, lung and kidney to reactive species, *N*-hydroxy-3-ABA, forming the same DNA adducts that are formed *in vitro* and *in vivo* in rodents by 3-ABA or 3-NBA (Arlt *et al.* 2003a, b, c, 2004b, 2005, 2006a, Stiborova *et al.* 2006a; 2008, 2009) (***Figure 1***).

In contrast to the enzymes activating 3-ABA to species binding to DNA, those participating in 3-ABA oxidation to other potential metabolites have not been extensively studied so far. Therefore, here we investigated the oxidative metabolism of 3-ABA *in vitro*, in order to characterize the 3-ABA metabolites and to identify CYPs responsible for their formation. Hepatic microsomal systems and recombinant CYP enzymes of rat, the organism found previously to be a suitable model mimicking activation metabolism of 3-ABA in human (Arlt *et al.* 2004b) were used for such a study. In addition, rat and human recombinant CYP enzymes were utilized to characterize their participation in 3-ABA oxidation.

MATERIAL AND METHODS

Synthesis of 3-ABA and N-hydroxy-3-ABA. 3-ABA and N-hydroxy-3-ABA were synthesized as described by Arlt *et al.* (2003a) and their authenticity was confirmed by UV spectroscopy, electrospray mass spectra and high field proton NMR spectroscopy.

Chemicals and enzymes. Microsomes from rat livers were isolated and characterized for CYP activities as described (Arlt *et al.* 2004b; Stiborova *et al.* 2006a,b). Supersomes™, microsomes isolated from insect cells transfected with Baculovirus constructs containing cDNA of one of the following rat CYPs: CYP1A1, 1A2, 2A1, 2A2, 2B1, 2C6, 2C11, 2C12, 2C13, 2D1, 2D2, 2E1, 3A1, 3A2 with cytochrome b_5 and expressing NADPH:CYP reductase and one of the following human CYPs: CYP1A1, 1A2, 2A6, 2B6, 2C8 (with and

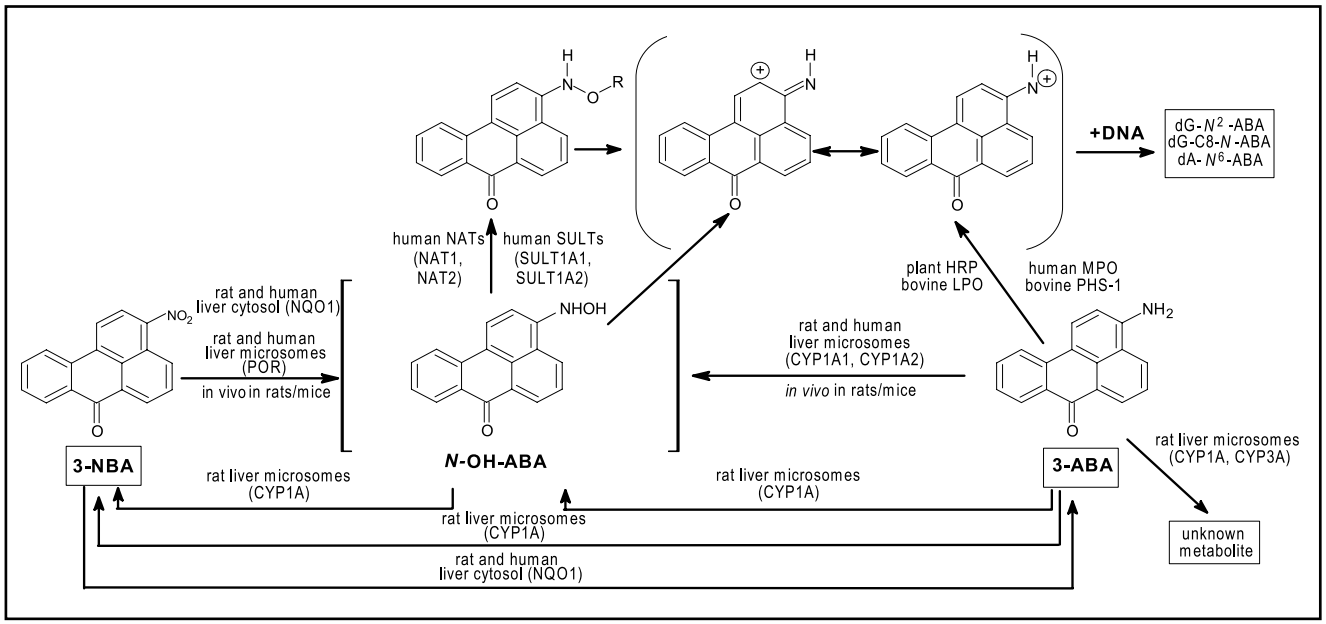

Figure 1. Pathways of metabolism and DNA adduct formation of 3-NBA and 3-ABA. See text for details. NQO1, NAD(P)H:quinone oxidoreductase; NAT, *N,O*-acetyltransferases; SULT, sulfotransferase; PHS-1, prostaglandin H synthase-1 (cyclooxygenase 1); CYP, cytochrome P450; LPO, lactoperoxidase; MPO, myeloperoxidase; POR, NADPH:cytochrome P450 oxidoreductase; R = -$COCH_3$ or -SO_3H. R = -$COCH_3$ or -SO_3H; dA-N^6-ABA, 2-(2′-deoxyadenosin-N^6-yl)-3-aminobenzanthrone; dG-N^2-ABA, *N*-(2′-deoxyguanosin-N^2-yl)-3-aminobenzanthrone; dG-C8-*N*-ABA, *N*-(2′-deoxyguanosin-8-yl)-3-aminobenzanthrone.

without cytcohrome b_5), 2C19 (with and without cytochrome b_5),, 2D6, 2E1, 3A4 and 3A5 (with and without cytcohrome b_5), and expressing NADPH:CYP reductase were obtained from Gentest Corp. (USA). NADPH, phenacetine, dimethylsulfoxide (DMSO) were obtained from Sigma Chemical Co (St Louis, MO, USA), ethylacetate, methanol for high performance liquid chromatography (HPLC) super gradient, methanol were obtained from Lachema, (Brno, Czech Republic).

Animal experiments and preparation of microsomes. The study was conducted in accordance with the Regulations for the Care and Use of Laboratory Animals (311/1997, Ministry of Agriculture, Czech Republic), which complies with the Declaration of Helsinki. Microsomes from livers of ten male untreated Wistar rats and those from livers of ten male rats pre-treated with β-naphtoflavone (β-NF) and phenobarbital (PB) were prepared by the procedure described previously (Arlt *et al.* 2004b; Stiborova *et al.* 2002b, 2006b; Krizkova *et al.* 2008; Sistkova *et al.* 2008). Protein concentrations in the microsomal fractions were assessed using the bicinchoninic acid protein assay with the bovine serum albumin as a standard (Weichelman *et al.* 1988). The concentration of CYP was estimated according to Omura and Sato (Omura & Sato, 1964) based on absorption of the complex of reduced CYP with carbon monoxide. Untreated rat liver microsomes contained 0.6 nmol CYP/mg protein. Hepatic microsomes of rats treated with β-NF and PB contained 1.3 and 1.5 nmol CYP/mg proteins, respectively.

Incubations. Unless stated otherwise, incubation mixtures used for studying 3-ABA metabolism by rat hepatic microsomes were prepared as described previously by Mizerovska *et al.* (2008). Briefly, incubation mixtures, containing final volume of 500 μl, consisted of 100 mM potassium phosphate buffer (pH 7.4), 10 mM NADPH, 0.5 mg of microsomal protein and 5–50 μM 3-ABA (dissolved in DMSO). The reaction was initiated by adding 3-ABA. Incubations with rat microsomes were carried out at 37 °C for 5 minutes. Control incubations were carried out either without the enzymatic system (microsomes) or without NADPH. Incubation mixtures used for studying 3-ABA metabolism by rat and human recombinant CYP enzymes contained final volume of 250 μl, consisting of 100 mM potassium phosphate buffer (pH 7.4), 10 mM NADPH, 1 μM CYP in Supersomes™ and 20 μM 3-ABA (dissolved in DMSO). All incubations were carried out at 37°C for 20 min. In the case of investigation of the time dependence of 3-ABA oxidation, reaction mixtures were incubated at 37°C for 0–120 minutes. Control incubations were carried out either without the CYP enzymes (Supersomes™) or without NADPH. Then, 2.5 or 5 μl of 1 mM phenacetine in methanol was added as an internal standard, and 3-ABA and its metabolites were extracted twice with ethyl acetate (2 × 1.5 ml). The extracts were evaporated to dryness; residues dissolved in 30 μl of methanol and subjected to reverse-phase (RP)-HPLC to evaluate the amounts of residual 3-ABA and its metabolites. The 3-ABA metabolites were separated from 3-ABA by HPLC with UV detection and characterized by mass spectrometry and co-chromatography with synthetic standards. The following chemicals were used in the inhibition studies of the 3-ABA oxidation by hepatic microsomes: α-naphthoflavone (α-NF), which inhibits CYP1A1 and 1A2; furafylline, which

Table 1. Kinetic parameters of 3-ABA oxidation catalyzed by hepatic microsomal CYPs

	Parameters	
Hepatic microsomes from rats pretreated with	K_m K_{m1} K_{m2} [μM]	V_{max} V_{max1} V_{max2} [nmol 3-ABA min^{-1} mg^{-1}]
β -naphtoflavone (CYP1A1/2)	47.5 ± 0.5	13.42± 0.134
Phenobarbital (CYP2B)	60.7 ± 0.6	16.8 ± 0.168
Control	20.7 ± 0.2 417.5 ± 4.18	3.4 ± 0,3 48.5 ± 4.85

Experimental conditions are described in Material and methods, 3-ABA (5 - 50 μM) and 0.5 mg microsomal protein were present in the incubation mixtures to determine the K_m and V_{max} values. Values in the table are averages and standard deviations of three determinations.

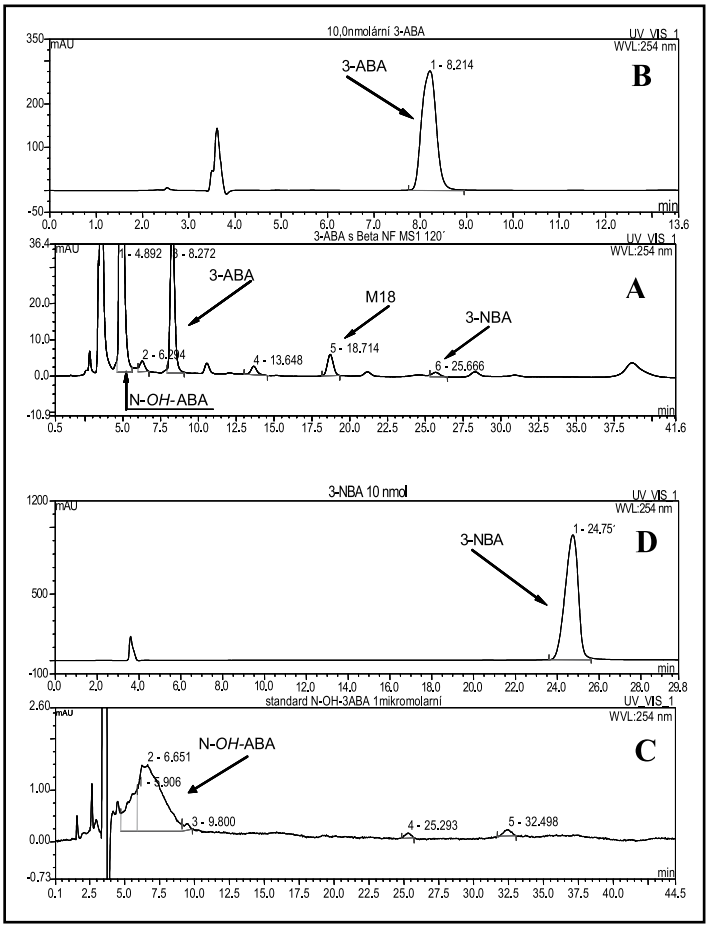

Figure 2. HPLC chromatographs of 3-ABA metabolites produced by hepatic microsomes of rats treated with β-NF (A), HPLC profiles of several standards; 3-ABA (B), *N*-hydroxy-3-ABA (C) and 3-NBA (D).

inhibits CYP1A2, diamantane, which inhibits CYP2B (Stiborova *et al.* 2002a); sulphafenazole, which inhibits CYP2C; diethyldithiocarbamic acid (DDTC), which inhibits CYP2E1 and ketoconazole, which inhibits CYP3A (Rendic & DiCarlo, 1997). Inhibitors were dissolved in ethanol, except of α-NF that was dissolved in a mixture of methanol:ethylacetate (3:2, v/v) and DDTC that was dissolved in distilled in water, to yield final concentrations of 1-1000 μM in the incubation mixtures.

HPLC. HPLC was performed with a reversed phase column (Nucleosil 100-5 C_{18}, Macherey-Nagel, Duren, Germany, 25 cm × 4.6 mm, 5 mm) proceeded by a C-18 guard column, using isocratic elution conditions of 70% methanol in distilled water with a flow rate of 0.6 ml/min. HPLC was carried out with a Dionex HPLC pump P580 with UV/VIS UVD 170S/340S spectrophotometer detector set at 254 nm, and peaks were integrated with a CHROMELEON™ 6.01 integrator. 3-ABA, *N*-hydroxy-3-ABA, and 3-NBA were eluted with retention times (r.t.) of 8.2, 6.5 and 25 minutes, respectively (***Figure 2***)

RESULTS

When 3-ABA was incubated with rat hepatic microsomes in the presence of NADPH up to three product peaks were separated by HPLC (see Figure 2 for hepatic microsomes of rats treated with β-NF). Using co-chromatography with synthetic standards, two of them were identified to be the products formed by oxidation of 3-ABA on its amino group back to the parent compound from which 3-ABA is generated in organisms, 3-NBA. Namely, *N*-hydroxylation metabolite, *N*-hydroxy-3-ABA and 3-NBA were identified to be these 3-ABA oxidation products. This finding indicates that 3-ABA might, in some cases, be oxidized through *N*-hydroxy-3-ABA back to its oxidative counterpart, 3-NBA (Figure 1). A structure of another metabolite eluted with the retention time (r.t.) of 18 min, M18 (Figure 2A) remains to be characterized.

We have used hepatic microsomes of rats treated with CYP inducers (an inducer of CYP1A1/2, β-NF and an inducer CYP2B1/2, PB), as well as inhibitors of individual CYPs, to evaluate the role of rat hepatic CYPs in 3-ABA metabolism (Mizerovska *et al.* 2008). Here, the same microsomal systems and hepatic microsomes of control (untreated) rats were utilized to determine the kinetics of 3-ABA metabolism. If 3-ABA was incubated with these microsomes more than 30 minutes, different pat-

terns of 3-ABA metabolic products were found in individual microsomes. Whereas hepatic microsomes rich in CYP1A1/2 (β-NF-microsomes) generated the final oxidation metabolite of 3-ABA, 3-NBA (Figure 1), this metabolite (3-NBA) was not formed by the other microsomes tested in the study (***Figure 3***). The metabolite with unknown structure, M18, was the only one formed from 3-ABA by these enzymatic systems (Figure 3). It should be mentioned that in some cases the control incubations without presence of microsomes contained traces of 3-ABA metabolites, 3-NBA and a metabolite M18 (see Figure 3A showing this case), which might probably be formed by autooxidation. *N*-hydroxy-3-ABA was another metabolite generated by microsomes rich in CYP1A1/2 (Figure 2A), but this metabolite was not quantified in this study, namely, because this reactive compound is easily decomposed and forms nitrenium and/or carbenium ion (Figure 1), being scavenged by proteins (at least partially) present in the incubation mixtures. When shorter incubation times were used in the experiments, formation of all 3-ABA oxidation products detected in the above experiments was low, being hardly to be quantified. Hence, because the incubation times during them the conversion of 3-ABA is linear (that are shorter than 30 minutes, see below) are needed to study kinetics of 3-ABA metabolism by microsomes, the consumption of 3-ABA, detected during such short incubation times, has been used to evaluate initial velocities of the enzymatic reactions. Conversion of 3-ABA by all three hepatic microsomes (measured by consumption of 3-ABA) was linear up to 5 minutes of incubation (data not shown). This time interval was, therefore, used to evaluate kinetics of 3-ABA oxidation.

A classical Michaelis-Menten kinetics was found for 3-ABA metabolism by hepatic microsomes of rats treated with β-NF and PB; double reciprocal plots of initial velocities *versus* concentrations of 3-ABA were linear (**Figure 4A,B**). The values of Michaelis constant (K_m) and maximum reaction velocity (V_{max}) determined from this kinetics are shown in ***Table 1***. The values of K_m and V_{max} for 3-ABA metabolism by PB-microsomes were more than 1.2-fold higher than those by hepatic microsomes of rats treated with β-NF.

In contrast to the kinetics found with rat hepatic β-NF- and PB-microsomes, double reciprocal plots of kinetics of 3-ABA oxidation by hepatic microsomes of control (untreated) rats were not linear (***Figure 4C***), giving two K_m values for 3-ABA (Table 1). These findings indicate that more than one of the CYP enzymes present in these microsomes are responsible for 3-ABA metabolism. Because one of the K_m values is close to the value of K_m found with microsomes rich in CYP1A1/2 (β-NF-microsomes), the enzyme of a CYP1A subfamily might be important for 3-ABA conversion in hepatic microsomes of control rats. Indeed, inhibitors of CYPs of a 1A subfamily (α-NF for CYP1A1/2 and furafylline for CYP1A2) are strong inhibitors of 3-ABA metabolism (***Table 2***). Results of experiments utilizing CYP inhibitors also suggest that CYP enzymes of a 3A subfamily might participate in 3-ABA metabolism in rat hepatic microsomes; a selective CYP3A inhibitor, ketoconazole, was highly effective

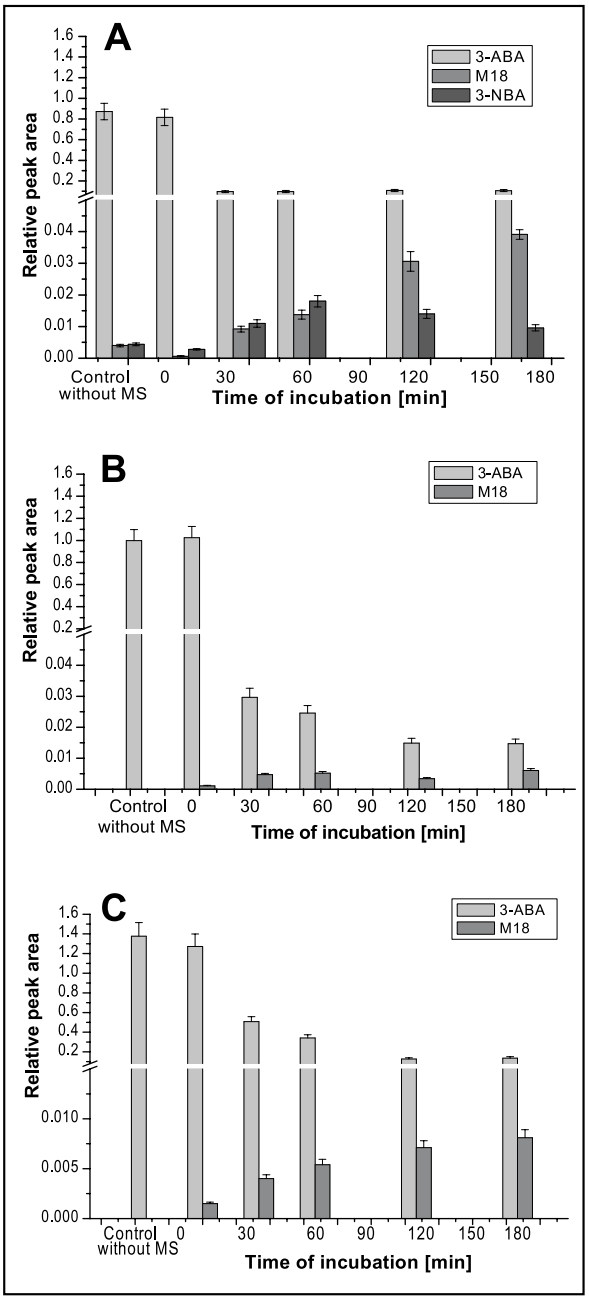

Figure 3. Time dependence of 3-ABA oxidation by hepatic microsomes of rats treated with β-NF (A), PB (B), and by those of control (uninduced) rats (C). Experimental conditions are described in Material and methods. Values of reaction rates of 3-ABA oxidation are averages and standard deviations of triplicate incubation. MS; microsomes

in inhibiting 3-ABA conversion, with the IC_{50} value of 3.5 μM (Table 2).

To further characterize the role of individual rat CYPs in 3-ABA metabolism and to compare their efficiencies with those of human CYP enzymes, microsomes of Baculovirus transfected insect cells (Supersomes™) containing recombinantly expressed rat and human CYPs and NADPH:CYP reductase were utilized in an additional part of this study.

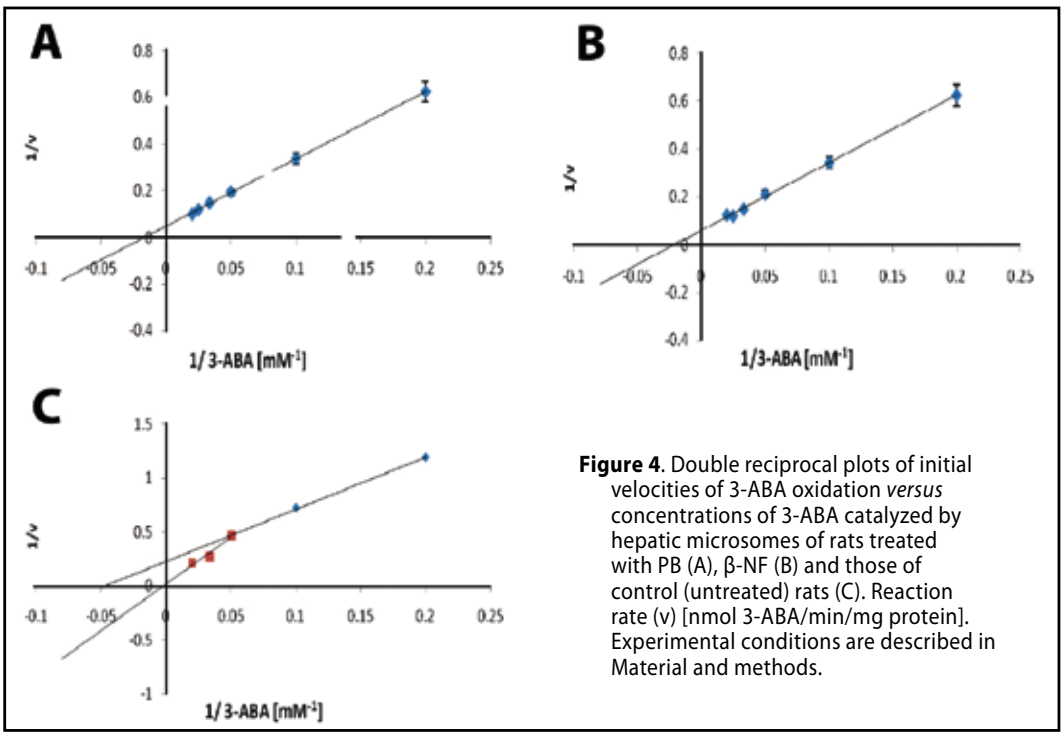

Figure 4. Double reciprocal plots of initial velocities of 3-ABA oxidation *versus* concentrations of 3-ABA catalyzed by hepatic microsomes of rats treated with PB (A), β-NF (B) and those of control (untreated) rats (C). Reaction rate (v) [nmol 3-ABA/min/mg protein]. Experimental conditions are described in Material and methods.

Table 2. The effects of CYP inhibitors on 3-ABA oxidation by rat hepatic microsomes

Hepatic microsomes from rats pretreated with[a]	Inhibitor[b]	IC_{50} [μM][c]
β-naphtoflavone (CYP1A1/2)	α-naphtoflavone (CYP1A1/2)	10.9 ± 1.0
β-naphtoflavone (CYP1A1/2)	Furafylline (CYP1A2)	16.6 ± 1.6
Untreated	Sulfaphenazole (CYP2C)	10.8 ± 1.0
Untreated	DDTC (CYP2E1)	96.8 ± 6.1
Untreated	Ketoconazole (CYP3A4)	3.5 ± 0.3

[a]Isoforms of CYPs induced by inducers are shown in brackets. [b]Isoforms of CYP inhibited by selective inhibitors are shown in brackets. [c]Estimated from concentration-dependent inhibition of 3-ABA oxidation by interpolation (inhibitors were 1 – 1000 μM depending on the chemical). 3-ABA (20 μM) and 0.5 mg microsomal protein were present in the incubation mixture. [d]Averages and standard deviations of three determinations.

First, a time-dependence of 3-ABA conversion catalyzed by rat recombinant CYP1A1, 3A1 and 3A2, the enzymes found in the above experiments to be important in 3-ABA metabolism in rat liver microsomes, and that of their human orthologous CYPs (CYP1A1 and 3A4) was investigated. Whereas 3-ABA conversion catalyzed by human and rat CYP1A1, rat CYP3A1 and human CYP3A4 is linear up to 20 minutes of incubations, that by rat CYP3A2 is linear even up to 40 minutes of incubation (data not shown). Because of linearity of 3-ABA conversion till 20 minutes, this time interval was used in experiments evaluating the efficiency of individual human and rat CYPs to metabolize 3-ABA.

All rat and human recombinant CYP tested in this study were effective to metabolize 3-ABA (***Figure 5***). Among them CYPs of a 3A subfamily (CYP3A2 and 3A4/5) followed by CYP1A1 were the most efficient enzymes of both organisms metabolizing 3-ABA. In addition, rat CYP2C11 and human CYP2A6, 2C19 and 2D6 were also effective in this reaction (Figure 5)

DISCUSSION

3-ABA, the human metabolite of the ubiquitous environmental pollutant 3-NBA, was detected in the urine of smoking and nonsmoking salt mining workers occupationally exposed to diesel emissions at similar concentration (1-143 ng/24 h urine) to 1-aminopyrene (2-200 ng/24 h urine), the corresponding amine of the most abundant nitro-polycyclic aromatic hydrocarbons detected in diesel exhaust matter (Seidel *et al.* 2002). Comparison between experimental animals and human CYP enzymes is essential for the extrapolation of animal toxicity data to assess human health risk. Therefore, here we compared the ability of rat and human CYP enzymes to metabolize 3-ABA.

The results found in the presented study have increased our knowledge on the potential of human and rat CYP enzymes to metabolize 3-ABA and on the kinetics of such reactions. Two experimental approaches were utilized in this study. For the first, we used hepatic microsomal systems of rat found to be a

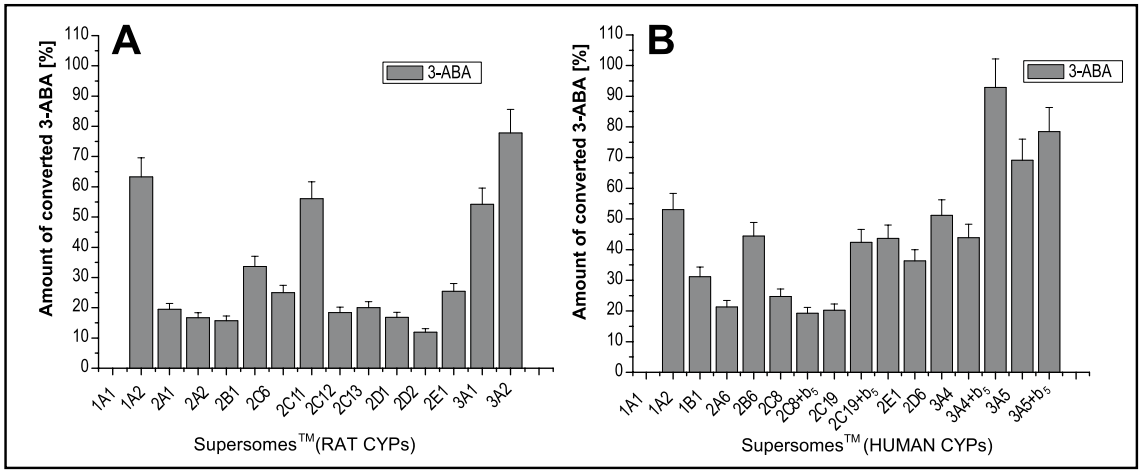

Figure 5. Oxidation of 3-ABA by recombinant CYPs of rat (A) and human (B). Experimental conditions are described in Material and methods. Values of reaction rates of 3-ABA oxidation are averages and standard deviations of triplicate incubation.

suitable experimental model (based on the fact that the same enzymes activate 3-ABA in human and rat livers to form DNA adducts) (Arlt *et al.* 2004b, 2006b, Stiborova *et al.* 2006a). Therefore, the results should provide some indications of what might occurs with 3-ABA in livers of human. The second approach employed rat and human recombinant CYP enzymes, which should mimic the enzymatic activity of actual CYP enzymes of both organisms.

Recently, were have found that rat and human hepatic CYP1A1 and 1A2, followed by human CYP2A6, are predominantly responsible for metabolic activation of 3-ABA to form DNA adducts. The extrahepatic enzymes CYP1B1 and 2B6 were also effective to activate 3-ABA, but to a much lower extent (Arlt *et al.* 2004b). On the contrary, none of the other CYP enzymes such as CYP2C9, 2D6, 2E1 and 3A4 tested in this former study activated 3-ABA to species generating DNA adducts (Arlt *et al.* 2004b). This finding corresponds to results found in the present study. We have found that CYP1A enzymes are effective in oxidation of 3-ABA to the final oxidative metabolite of this substance, 3-NBA. Namely, to the metabolite that is formed through the formation of *N*-hydroxy-3-ABA. This reactive intermediate (N-hydroxy-3-ABA) found in this study to be formed by CYP1A enzymes, is also decomposed to the ultimate carcinogenic species of 3-ABA, nitrenium and/or carbenium ions, forming the 3-ABA-derived DNA adducts (Figure 1). Hence, this metabolic activation pathway seems to be mediated mainly by the CYP1A enzymes. On the contrary, 3-ABA oxidation to further product(s) such as metabolite M18, which is supposed to be a detoxification metabolite (Mizerovska *et al.* 2008), is catalyzed not only by CYP1A, but also by other rat CYP enzymes. As follows from the studies on kinetics of 3-ABA metabolism (presented study) as well as from the effects of selective inhibitors of individual CYPs (Mizerovska *et al.* 2008 and presented study), beside CYP1A, the CYP enzymes of 3A and 2C subfamilies might be very effective in such 3-ABA metabolism in rat livers. Using rat recombinant CYPs, this suggestion was confirmed; rat CYP3A2 followed by CYP1A1, 2C11 and 3A1 were the most efficient in 3-ABA conversion. The results of this work also support our former findings showing a suitability of the rat as an appropriate animal model to study the fate of 3-ABA in humans (Arlt *et al.* 2004b; Stiborova *et al.* 2006a). Namely, here we have found that human CYPs of a 3A subfamily (CYP3A and 3A5), orthologous CYPs to rat enzymes mentioned above, followed by human and rat CYP1A1, are the most efficient in 3-ABA metabolism.

In conclusion, the present study characterizing two metabolites formed from 3-ABA by CYP-mediated oxidation, namely, a metabolite of 3-ABA, which is responsible for generation of 3-ABA-derived DNA adducts (N-hydroxy-3-ABA) and its final oxidation product (3-NBA), confirmed the participation of CYP1A enzymes in metabolic activation of 3-ABA (Arlt *et al.* 2004b). Other rat and human CYP enzymes, such a CYP3A and 2C are mainly important for detoxification metabolism of this compound. Structural characterization of the major detoxification metabolite of 3-ABA awaits further investigation.

Acknowledgment

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