METABOLIC ACTIVATION/INACTIVATION

Polycyclic aromatic hydrocarbon (PAH) metabolizing enzyme activities in human lung, and their inducibility by exposure to naphthalene, phenanthrene, pyrene, chrysene, and benzo(*a*)pyrene as shown in the rat lung and liver

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Abstract In order to survey changes and activities in the polycyclic aromatic hydrocarbon (PAH)-metabolizing enzymes implicated in lung cancer susceptibility studies, we investigated enzyme induction by 2–5-ring-sized 'biomarker' PAHs in rat liver and lung, and the activities in five human lung specimens. Naphthalene, phenanthrene, pyrene, chrysene, and benzo[*a*]pyrene (B*a*P) were administered to rats for 3 days (25–128 mg/kg/day) and the responses compared with those of model inducers. PAH treatment increased the CYP1A-catalyzed activity of pyrene 1-hydroxylation and 7-ethoxyresorufin *O*-deethylation in rat liver by up to 28- and 279-fold, and in rat lung by up to 22- and 51-fold, respectively. 1-Naphthol (hUGT1A6), 1-hydroxypyrene (hUGT1A6/1A9), and entacapone (hUGT1A9) are markers of PAH-glucuronidating human uridine diphosphate-glucuronosyltransferases (UGT). These activities increased up to 6.4-fold in rat liver and up to 1.9-fold in rat lung. NADPH:quinone oxidoreductase 1 (NQO1) and glutathione *S*-transferase activities increased up to 5.3- and 1.6-fold (liver), and up to 4.4- and 1.4-fold (lung), respectively. CYP1A showed the best liver-to-lung relationship ($R^2 = 0.90$). The inducing efficiency by PAHs differed extensively: control $\leq$ naphthalene < phenanthrene, pyrene << chrysene < B*a*P. In human lung (non-smokers), the marker activities of CYP1A1, UGT1A6/1A9, and NQO1 were lower than those in rat lung. Epoxide hydrolase activity was 1,000-fold higher than the pulmonary CYP1A1 activities. Human UGT and NQO1 displayed large variations (>60-fold), many times greater than the experimental (inducible/constitutive) variation in the rat. Kinetics of 1-hydroxypyrene glucuronidation showed two low-K_m forms both in rat and human lung. Since the 2–4-ring PAHs (major constituents) were poor enzyme inducers, it appears that the PAH-metabolizing pathways are mainly induced by B*a*P-type minor constituents. Gene–environmental interactions which magnify polymorphic variability in pulmonary bioactivation/detoxification capacity probably play a key role in individual susceptibility to (or protection against) chemically induced lung cancer. Hence, human exposure to PAH mixtures with high content of B*a*P-type hydrocarbons confers a potentially higher health risk than PAH mixtures with low content of procarcinogens.

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UGT kinetics · Human lung · Rat lung and liver ·
Individual susceptibility

Abbreviations

ALT	Alanine aminotransaminase
ANF	α-Naphthoflavone
BNF	β-Naphthoflavone
B*a*P	Benzo[*a*]pyrene
BSO	L-Buthionine-[*S,R*]-sulfoximine
CDNB	1-Chloro-2,4-dinitrobenzene
CYP or P450	Cytochrome P450
DCPIP	2,6-Dichlorophenolindophenol
EQ	Ethoxyquin
EROD	7-Ethoxyresorufin *O*-deethylase
E-UGT	Entacapone UGT
GST	Glutathione *S*-transferase
HPLC	High-performance liquid chromatography
MC	3-Methylcholanthrene
mEH	Microsomal epoxide hydrolase
NQO1	NADPH:quinone oxidoreductase 1 [NMO1, quinone reductase, DT-diaphorase]
NP-oxide (EPNP)	1,2-Epoxy-3-(*p*-nitrophenoxy) propane
NP-diol	3-[*p*-Nitrophenoxy]-1,2-propane diol
N-UGT	1-Naphthol UGT
1-OHP	1-Hydroxypyrene
PH	Pyrene 1-hydroxylase
PAH	Polycyclic aromatic hydrocarbon
UDP	Uridine diphosphate
UDPGA	UDP-glucuronic acid
UGT	UDP-glucuronosyltransferase

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic environmental pollutants arising from incomplete combustion or pyrolysis of organic material. Exposure to PAHs has been associated with adverse effects on human health, including the risk of cancer (ACGIH 2005; IARC 1985). Humans are exposed to PAHs via a variety of sources such as diet, tobacco smoking, and environment (Hecht 2002). Of greatest concern is, however, the health of employees in factories and other areas where PAH measurements show high uptake levels. The body burden of PAH workers may well exceed by 10–1,000 times the values of non-occupationally exposed people (Grainger et al. 2004; Huang et al. 2004; UBA 1998; Yang et al. 1999). In practice, the internal exposure levels are assessed by biological monitoring.

Urinary metabolites of naphthalene, phenanthrene, chrysene, pyrene, and benzo [*a*]pyrene (B*a*P) are biomarkers of PAH exposure, and for this reason, studies into these PAHs are of interest to occupational exposure and hazard assessment (Angerer et al. 1997; Grimmer et al. 1997). The first four of these compounds belong to the main constituents of PAH mixtures, whereas B*a*P denotes a much smaller but hazardous fraction of poorly volatile (particulate), large molecular size substances in which B*a*P and many other compounds are procarcinogens (ACGIH 2005; Bouchard and Viau 1999; Elovaara et al. 1995a).

Drug and carcinogen metabolizing enzymes are regarded as one class of environmental susceptibility genes (Guengerich 2000; Pelkonen and Nebert 1982; Pelkonen et al. 1999; Thier et al. 2003). In the case of lung cancer, polymorphism in metabolic genes confers a much higher risk in smokers than in non-smokers, indicating that genetic polymorphism per se is not crucial in the absence of carcinogenic exposure (Vineis 2004). The complex relationship between polymorphism, carcinogen dose, and lung cancer is influenced by enzyme induction of pathways which biotransform PAH procarcinogens into highly mutagenic diol epoxides, radical cations, and *o*-quinones (Conney 1982; Gelboin 1980; Penning et al. 1999). Many of the cancer susceptibility enzymes are coordinately inducible proteins. This means that the intraindividual tissue activity levels may vary multifold due to gene–environmental interactions.
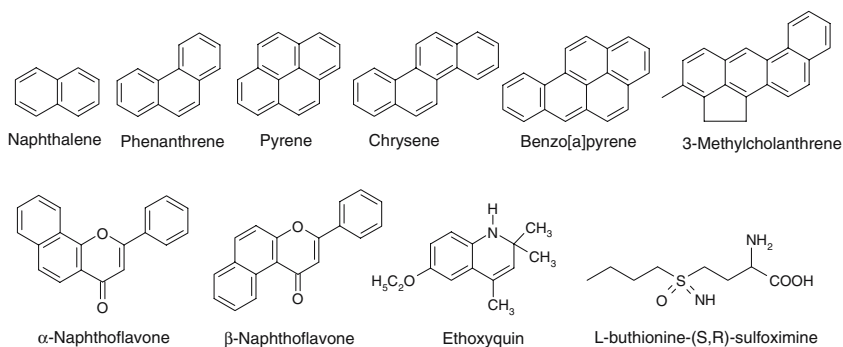
The following PAH metabolizing 'susceptibility' enzymes were studied in this work: CYP1A1 (Nebert et al. 2004; Shimada et al. 1992; Shimada and Fujii-Kuriyama 2004), microsomal epoxide hydrolase (mEH) (Gelboin 1980; Kiyohara et al. 2002), NADPH:quinone oxidoreductase 1 (NQO1) (Jaiswal 2000; Joseph and Jaiswal 1994), uridine diphosphate (UDP)-glucuronosyltransferase (UGT) (Burchell et al. 1998; Bock et al. 1998; Zheng et al. 2002), and glutathione *S*-transferase (GST) (Hayes et al. 2005). CYP1A1 is inducibly expressed in human lung (Hukkanen et al. 2002; Kim et al. 2004a). Both 7-ethoxyresorufin *O*-deethylase (EROD) and pyrene 1-hydroxylase (PH) are markers of CYP1A1 activity. Pyrene is hydroxylated to 1-OHP (1-hydroxypyrene) mainly by CYP1A1 in lung microsomes from smokers (Elovaara et al. 1995b). This reaction is highly catalyzed by CYP1A1, less by CYP1B1 and CYP1A2, while 2A6, 2C8, 2C9*1, 2C19, 2D6, 2E1, and 3A4 show little activity (Kim et al. 2004b). 1-Naphthol, 1-OHP, and entacapone are sensitive substrates for detecting UGT1A induction (Elovaara et al. 2004; Luukkanen et al. 1997). These are markers for hUGT1A6, hUGT1A6/1A9, and hUGT1A9, respectively. Also, other human UGT1A forms can glucuronidate 1-OHP and entacapone

(Luukkanen et al. 2005). B*a*P, 3-methylcholanthrene (MC), β-naphthoflavone (BNF) are typical inducers of Ah receptor (AhR) regulated gene expression (Bock et al. 1998; Nioi and Hayes 2004). α-Naphthoflavone (ANF) is a prototype flavonoid with inhibitory (CYP1A1) and stimulatory (CYP3A4) effects on B*a*P metabolism (Koley et al. 1997; Shou et al. 1994). L-Buthionine-[*S,R*]-sulfoximine (BSO) is an inhibitor of glutathione synthesis and a selective inducer of phase II enzymes (Manning and Franklin 1990). Ethoxyquin (EQ) is a phenolic antioxidant and monofunctional inducer of NQO1, GST, and UGT1A6 (Buetler et al. 1995; Nioi and Hayes 2004).

Polycyclic aromatic hydrocarbons in tobacco smoke induce CYP1A enzyme activities in human lung (Anttila et al. 2001; Kim et al. 2004a; Vainio et al. 1995), liver (Pelkonen et al. 1986), and placenta (Myllynen et al. 2005). A high degree of work-related exposure to PAHs probably also results in enzyme induction, particularly in employees who smoke. Occupational exposure measurements show that the uptake of PAHs known as potent inducers (B*a*P) is relatively very low (Gundel et al. 2000). Hence, the B*a*P dose alone may not evoke any marked enzyme induction in human lung. The question is do the more abundant but less hazardous 2–4-ring sized PAHs act as coinducers of enzymes that activate/detoxify procarcinogens in PAH mixtures.

Since susceptibility to or protection against chemical carcinogenesis is partially determined by differences in the biotransformation of procarcinogens, we used a rat model to evaluate the hepatic and pulmonary enzyme induction of carcinogen metabolizing phase I and II activities in animals treated with the PAH-mixture marker substances naphthalene, phenanthrene, pyrene, chrysene, and B*a*P (see Fig. 1). The inducibility by these 2–5-ring PAHs was compared with that by ANF, BNF, MC, EQ, and BSO. The sensitivity of our methods enabled us to quantify the enzyme activities CYP1A1, NQO1, mEH, and UGT in five human lungs and compare the human and animal data. The enzyme kinetics of 1-OHP glucuronidation (1-OHP UGT) could be determined in rat and human lung microsomes.

Materials and methods

Chemicals

Naphthalene (99%), phenanthrene (96–98%), pyrene (98%), chrysene (98%), B*a*P (> 97%), and MC (98%), 1-naphthol; 1-naphthyl β-D-glucuronide (sodium salt, 99% pure), ANF, BNF (95%), EQ (75%), 1,2-epoxy-3-(*p*-nitrophenoxy)propane (NP-oxide), 3-(*p*-nitrophenoxy)-1,2-propane diol (NP-diol), uridine 5′-diphosphate-glucuronic acid (UDPGA, triammonium salt), 7-ethoxyresorufin, and bovine serum albumin were purchased from Sigma-Aldrich (St Louis, MO, USA, or Steinheim, Germany). 1-OHP was from Acros Organics (Geel, Belgium). Entacapone [(E)-2-cyano-*N,N*-diethyl-3-(3,4-dihydroxy-5-nitrophenyl) propenamide; OR-611] was kindly provided by Orion Pharma (Espoo, Finland). 1-Pyrenyl β-D-glucuronide and entacapone 3-*O*-β-D-glucuronide were synthesized in the University of Helsinki (Luukkanen et al. 2001). The purity of the latter substances was ≥ 97.5%. Other reagents were of the highest grade available.

Animals and study design

Sixty female Wistar rats (298 ± 32 g, from the Helsinki University Breeding Centre) were housed in plastic cages bedded with aspen wood shavings in an air-conditioned room (23–25°C) with artificial lighting from 07:00 to 19:00 h. They had free access to the pellet chow (Rat Chow R3, Astra Ewos Ab, Södertälje, Sweden) and tap water. Four or eight rats per group were treated for 3 days with the doses shown for naphthalene (1.0 mmol/kg on day 1; 0.50 mmol/kg on day 2 and 3); phenanthrene, pyrene, chrysene (0.50 mmol/kg); B*a*P, MC (0.10 mmol/kg, ip); ANF, BNF (0.24 mmol/kg, ip); EQ (1.0 mmol/kg, ig); and with BSO (0.9 mmol/kg, ig) which was administered in tap water (100 mg/2.5 ml/kg body weight, twice a day, ig). All other agents were administered in olive oil (3 ml/kg). The first five substances were given both by oral gavage (day 1) and

Fig. 1 Structures of the individual PAHs and model inducer compounds used in the rat study

Naphthalene   Phenanthrene   Pyrene   Chrysene   Benzo[a]pyrene   3-Methylcholanthrene

α-Naphthoflavone   β-Naphthoflavone   Ethoxyquin   L-buthionine-(S,R)-sulfoximine

by ip administration (day 2 and 3). Inducer and vehicle-treated control animals were killed 24 h after the third dose. Food was withdrawn 3 h before each treatment and before sacrifice. Blood (6–8 ml) was drawn under CO_2 anesthesia by cardiac puncture with heparin-rinsed plastic syringes and transferred in plastic tubes (in an ice bath). The livers were perfused in situ with phosphate buffered saline, removed, weighed, cooled in ice, and stored frozen ($-70°C$). The experiments were approved by the local Ethical Committee for Animal Studies (the guidelines of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes were followed).

Human lung specimens

Human lung samples were obtained from the University Hospital of Oulu. All donors (two females and three males, age 25–71 years) were non-cancerous lung surgery patients, current non-smokers diagnosed with emphysema, atelectasis (perhaps hereditary), or granulomatous lesions (post tuberculosis). Use of the lung tissue was approved by the Ethics Committee of the Medical Faculty of the University of Oulu, Finland. The samples were placed on ice after the surgical excision, cut into pieces, frozen in liquid nitrogen, and stored at $-80°C$ until use. Microsome and cytosol fractions were prepared by differential ultracentrifugation and the protein determined as described (Nakajima et al. 1995), and then stored at $-70°C$ until biochemical analyses.

Preparation of microsomes and cytosol

The thawed livers and lungs were homogenized in three volumes (w/v) of cold 0.15 M KCl–50 mM Na–K–phosphate buffer (pH 7.4) with a Potter–Elvehjem glass homogenizer (Thomas tubes with Teflon pestle). Enzyme fractions were prepared at 5°C by differential ultracentrifugation. The supernatant of the first centrifugation (10,000 g, 15 min) was run at 106,000 g for 60 min to obtain the cytosol fraction as a supernatant (stored at $-70°C$). The microsomal pellet was suspended in 3 or 6 ml (lung/liver) of buffer, and centrifuged (106,000 g, 60 min). The washed microsomal pellet was briefly homogenized (resuspended) in the freezing buffer containing 10% glycerol to give a protein content of 10–20 mg/ml, and divided in aliquots which were stored at $-70°C$ until use. The protein concentrations were determined (Lowry et al. 1951) using commercial protein standards (Preciset® Eiweiss/Protein, Boehringer Mannheim, Mannheim, Germany).

Enzyme activity analyses

Alanine aminotransaminase (ALT) was determined in blood plasma (obtained by centrifugation 3,000 g for 20 min, at 20°C) using enzyme reagent kits from Medix Biochemica (Kauniainen, Finland). EROD activity was determined by a fluorometric method (Prough et al. 1978) in human lung microsomes (Anttila et al. 2001) and in rat lung and liver microsomes (Elovaara et al. 1991). mEH was determined by quantitative high-performance liquid chromatography (HPLC) methods as described previously (Nakajima et al. 1995). Briefly, the mEH activity toward 1.7 mM NP-oxide was determined by analysis of the product NP-diol (at 315 nm) or the activity toward 0.3 mM styrene oxide by analysis of the product styrene diol (at 215 nm) by an HPLC method (Giuliano et al. 1980). The NQO1 activity was measured kinetically at 30°C (Ernster 1967). Briefly, the dicoumarol (0.010 mM)-sensitive reduction of 2,6-dichlorophenolindophenol (0.04 mM DCPIP) was followed at 600 nm using NADPH (0.1 mM) as the reductant, cytosolic protein from rat liver or lung (2–20 μg), or human lung (200–320 μg), 40 mM Tris–HCl buffer, pH 7.5, and 0.08% Triton X-100 in a final volume 2.1 ml (or 1.2 ml). The reaction was started with NADPH after a 5-min preincubation at 30°C. The activity was calculated by the molar absorption coefficient 20.8 mM^{-1} cm^{-1} for DCPIP. The GST activity toward 1-chloro-2,4-dinitrobenzene (CDNB) was recorded kinetically at 25°C with a spectrophotometric method (Habig et al. 1974). Briefly, the reaction mixture (2.5 ml) contained 1.0 mM CDNB, 1.2 mM GSH, 0.02% Triton X-100 in 90 mM potassium phosphate buffer (pH 6.5), and cytosolic protein from rat liver (4–14 μg) or lung (17–60 μg).

Pyrene hydroxylation

The reaction mixture (final volume 250 μl) contained microsomal protein from rat liver (5–10 μg) or lung (70–200 μg) with 0.15 M KCl–50 mM Na–K–phosphate buffer (pH 7.4), and pyrene (94 μM, added in dimethylsulfoxide). The reaction was started (after a 6-min preincubation at 37°C) with 25 μl of 10 mM NADPH (1 mM), incubated for 10 min at 37°C and stopped with 50 μl of ice-cold 15% $ZnSO_4$. After a 15 min protein precipitation (at 4°C), 1 ml acetonitrile was added to the tubes, mixed, and centrifuged (10,000 rpm, 10 min, 20°C). A 20 μl aliquot of the obtained supernatants was analyzed with a reversed phase C_{18} column (Waters Spherisorb S3 ODS2, 4.6 mm × 100 mm) and a Waters Spherisorb S5 ODS2 (4.6 mm × 30 mm) guard column in a Shimadzu HPLC/Class VP 5.021 chromatography system (Shimadzu, Kyoto, Japan) fitted with two LC-10AD

pumps, an SCL-10AVP system controller, an SIL-10A autoinjector and an RF-10A fluorescence detector (λ_{ex} = 242 nm, λ_{em} = 388 nm). 1-OHP (RT = 2.8 min) and pyrene (RT = 6.8 min) were eluted with 75% acetonitrile in 0.5% aqueous acetic acid at a flow rate of 1.1 ml/min. 1-OHP was quantitated by peak area calculation with a 7-point (0–600 nM) linear calibration curve ($r > 0.99$). The enzyme reaction was linear with incubation time and protein concentration.

1-OHP glucuronidation

The reaction mixture contained 25 µl of rat liver microsomes (0.3–3 µg protein) in 0.5% bovine serum albumin; 115 µl of 50 mM K/Na–phosphate buffer–0.15 M KCl (pH 7.4) with 10 mM MgCl$_2$; 100 µl of 50 mM K/Na–phosphate buffer–0.15 M KCl (pH 7.4) with 11 mM UDPGA (samples) or without UDPGA (controls). The reaction was started (after a 5–7 min preincubation at 37°C) with 10 µl of 150 or 7,500 µM 1-OHP in dimethylsulfoxide, incubated for 10 min, stopped with 50 µl of cold 15% ZnSO$_4$ solution, cooled for 15 min in an ice bath (protein precipitation) before addition of 500 µl of acetonitrile. The tubes were vortex-mixed (10 s), sonicated (2 min), and centrifuged (10,000 g, 10 min, at 20°C). The supernatants were analyzed as described previously (Luukkanen et al. 2001) in a Shimadzu HPLC/Class VP 5.021 chromatography system (Shimadzu) using an RF-10Axl fluorescence detector (λ_{ex} = 242 nm, λ_{em} = 382 nm) and 60% acetonitrile in a 0.5% aqueous acetic acid for isocratic reverse-phase C$_{18}$ runs. 1-Pyrenyl β-D-glucuronide was used for product identification (RT = 2.5 min) and quantitation by 6-point (0–56 nM) linear calibration curves ($r \geq 0.99$) and peak area calculation. The enzyme reaction was linear with incubation time ($\leq$ 30 min) and protein concentration (0.3–4 µg/250 µl).

Entacapone glucuronidation

The incubation mixture (final volume 250 µl, in Eppendorf tubes) contained 50 mM phosphate buffer (pH 7.4), 3 mM MgCl$_2$, 250 µM entacapone (added in assay buffer), and 20–50 µg (rat liver) or 50–100 µg (rat lung) microsomal protein. The reaction was started with 50 µl of 25 mM UDPGA (5 mM), incubated for 15 min at 37°C, and stopped with 25 µl of cold 4 M perchloric acid. After protein precipitation (10 min in ice-bath) the tubes were mixed and centrifuged (14,000 rpm, 5 min). The supernatants (liver) were analyzed in a Shimadzu HPLC/Class VP 5.021 chromatography system (Shimadzu) equipped with a Nova-Pak C18 column (4 µm, 150 mm × 3.9 mm; Waters Co., Milford, MA, USA), SPD-10AV UV-VIS detector (at 305 nm)

and an SIL-10A autoinjector. Acetonitrile—0.5% acetic acid in Millipore H$_2$O (25:75, v/v) was used as the mobile phase for isocratic runs (flow rate 1 ml/min) and calibration was based on a 7-point (0.4–4.0 µM) concentration curve ($R^2 > 0.99$). The lung activities were analyzed with an Agilent 1100 series HPLC with the column switching technique as reported by Keski-Hynnilä et al. (2001). Briefly, 25 µl of the internal standard (600 ng/ml tolcapone glucuronide) was added to the lung samples. Two hundred and fifty microliters of the supernatant was neutralized with 10 µl of 4 M NaOH before analysis. Entacapone glucuronide was quantitated with a Sciex API300 triple quadrupole mass spectrometer (Sciex, Concord, Canada) equipped with an electrospray ion source. The loss of the glucuronic acid moiety was monitored in the negative ion mode. Calibration was based on a 7-point (38–379 ng/ml) concentration curve ($R^2 > 0.99$). Entacapone 3-*O*-β-D-glucuronide was used for the product identification and quantitation by peak area calculation. The enzyme reactions were linear with incubation time (< 60 min) and protein concentration (10–100 µg/250 µl).

1-Naphthol glucuronidation

This activity was determined at 37°C (10 min incubation) in a 250-µl reaction mixture, containing microsomal protein from rat lung (15–41 µg) or liver (38–91 µg), and a 250 or 500 µM 1-naphthol concentration, respectively. Otherwise the reaction conditions were as in the 1-OHP UGT assay. The supernatants of the lung (1:11, v/v) and liver (1:31, v/v) were diluted in 30% acetonitrile–0.5% aqueous acetic acid (eluent) for isocratic analysis (5 µl injection, flow rate 0.9 ml/min) with a reverse-phase C$_{18}$ guard cartridge (Spherisorb S5 ODS2, 30 mm × 4.6 mm) and an analytical column S3 ODS2, 150 mm × 4.6 mm (Waters Spherisorb, Wexford, Ireland) using an RF-10Axl fluorescence detector (λ_{ex} = 290 nm, λ_{em} = 332 nm) and an SIL-10ADvp auto injector in a Shimadzu HPLC/Class VP 6.12 SP2 chromatography system. 1-Naphthol β-D-glucuronide was used for product identification (RT = 4.1 min) and quantitation by 6-point linear calibration curves (0.1–1.5 µM; $r = 1.00$) and peak area calculation. The enzyme reaction was linear with incubation time ($\leq$ 20 min; protein 150 µg/250 µl) and protein concentration (lung, 13–80 µg/250 µl; liver, 37–300 µg/250 µl; 10 min incubation).

Enzyme kinetics

Apparent kinetic constants (K_m and V_{max}) were determined by fitting the initial glucuronidation velocities (V) and substrate (S) concentrations to the Michaelis–

Menten equation by a non-linear least-squares method (Leonora v1.0, Cambridge University Press, UK). The Eadie–Hofstee plotting method (V versus V/S) was applied to explore the biphasic nature of 1-OHP UGT kinetics.

Statistical analyses

Rat liver and lung enzyme activity values were LN transformed to compute the coefficient of determination (R^2) by simple bivariate linear regression statistics (Table 2). Twelve of the 57 scatter plots are shown in Fig. 2 to illustrate some typical features observed in enzyme activity correlation behavior. Student's t-test was used for statistical analysis of rat group results.

Results

Inducibility of PAH-metabolizing enzyme activities in rat liver and lung

The 3-day treatments with single PAHs and model enzyme inducers were well tolerated by the rats, only after the first naphthalene dose (1.0 mmol/kg) did the rats exhibit nausea, but not after the dose was halved on day 2 and 3. The liver weights were slightly increased by most treatments. There were no significant effects on the lung or kidney organ weights, or the plasma ALT levels (data not shown).

The effects of exposure to the PAHs naphthalene, phenanthrene, pyrene, chrysene, and BaP are compared in Table 1. The CYP1A1 marker activity of EROD and PH increased in rat liver up to 279- and 28-fold, the corresponding increases in rat lung being up to 56- and 22-fold, respectively. The glucuronidation rate of 1-naphthol (1N-UGT), 1-hydroxypyrene (1-OHP UGT), and entacapone (E-UGT) increased up to 6.4-fold in rat liver and up to 1.9-fold in rat lung. NQO1 and GST activities increased up to 5.3- and 1.6-fold (liver), and up to 4.4- and 1.4-fold (lung), respectively. Despite the multifold CYP1A induction, the tissue-specific activities of EROD and PH increased less than the UGT, NQO1, or GST activities. The hepatic and pulmonary induction profiles were similar. The inducing efficiency by naphthalene, phenanthrene, pyrene, chrysene, and BaP was dependent on the PAH molecular size and structure. NQO1 was the only activity inducible by all individual PAHs, i.e., also by naphthalene. Otherwise this 2-ring PAH proved ineffective, while the effects by BaP were comparable with those evoked by BNF, MC, BSO + MC, or EQ + MC.
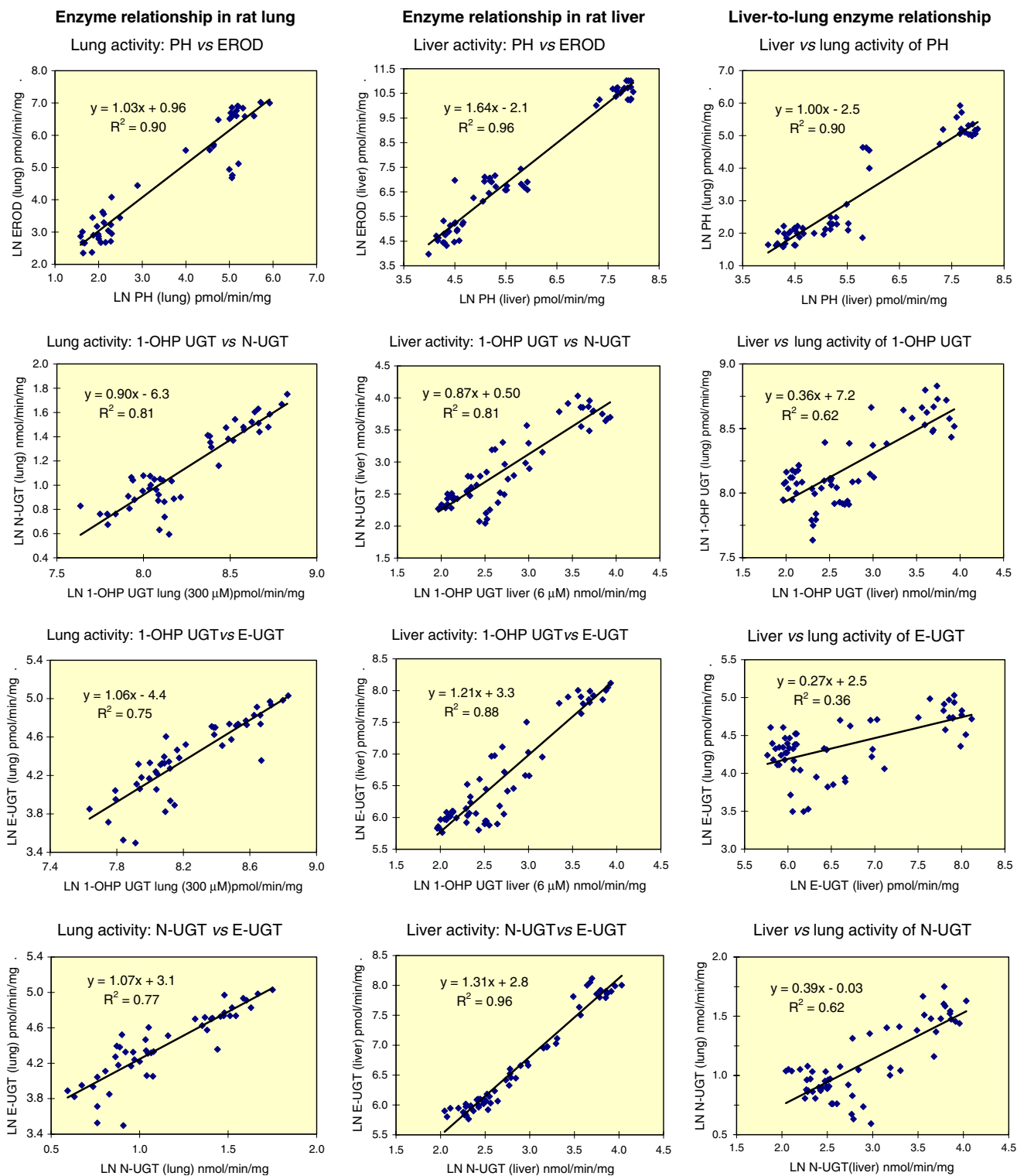
The hepatic activities of EROD, PH, N-UGT, 1-OHP UGT, E-UGT in control rat microsomes were about 9, 11, 4, 19, and 6 times greater than the corresponding pulmonary activities (Table 1). The GST activity was eight times greater in the liver than in the lung. The hepatic and pulmonary levels of NQO1 were similar in 9 of 13 subgroups. Only the groups treated with MC, BSO + MC, EQ, or EQ + MC exhibited a 2–3-fold greater activity in the liver than in the lung. BaP treatment enhanced the hepatic glucuronidation of 1-naphthol, 1-OHP, and entacapone 4.8-, 2.1-, and 6.4-fold, respectively, but less than twofold the glucuronidation in the lung. Chrysene was also an inducer of these UGTs, while naphthalene, phenanthrene, and pyrene were ineffective.

Hepatopulmonary relationships between phase I and II enzyme activities

The phase I and II enzyme activities in rat lung and liver were all inducible (Table 1). Linear regression statistics revealed that the relationships between EROD, PH, N-UGT, 1-OHP UGT, E-UGT, and NQO1 activities in the liver ($R^2 = 0.66$–0.96) and the lung ($R^2 = 0.46$–0.90) were similar (Table 2). These activities correlated less well with the overall GST activity in rat liver ($R^2 = 0.40$–0.77) and lung ($R^2 = 0.13$–0.39, $P \leq 0.02$). The R^2 value (0.13–0.96) describing a weak to strong relationship between the hepatic and pulmonary activities increased in the order: GST $\ll$ UGT1A < NQO1 < CYP1A (PH, EROD). In Fig. 2, the plots for the CYP1A marker activities (60 rats) showed a strong linear relationship between PH and EROD both in the liver and the lung ($R^2 = 0.90$–0.96). Also for the UGT1A marker activities, a good linear relationship was shown both in the liver and the lung ($R^2 = 0.75$–0.96). A strong linear relationship was found even between the liver-to-lung levels of PH, denoting CYP1A activity ($R^2 = 0.90$). The R^2 values for the liver-to-lung levels of the tested UGT1A activities ranged from 0.36 to 0.62 (Fig. 2). AhR regulated tissue activities (CYP1A1, UGT1A, NQO1) correlated with each other better ($R^2 = 0.46$–0.96) than with the GST activity ($R^2 = 0.13$–0.72).

PAH-metabolizing enzyme activities in human lung of non-smokers

Pyrene hydroxylation was 63–153-fold slower, and that of 1-OHP glucuronidation 2–171-fold slower in human lung microsomes than the (mean) pulmonary rates in BaP-induced rats (Tables 1, 3). Activity of mEH both toward styrene oxide and NP-oxide was high in human

Enzyme relationship in rat lung

Enzyme relationship in rat liver

Liver-to-lung enzyme relationship

Fig. 2 Relationships between PAH-metabolizing phase I and II enzymes activities, as shown for the two CYP1A marker activities, PH and EROD, and the glucuronidation activities toward N-

UGT, 1-OHP UGT, and E-UGT in rat lung and liver (*left and middle panels*, respectively). Correlations between the hepatic and pulmonary enzyme activity are shown in the panels to the *right*

lung microsomes. The mEH levels were 3–4 orders of magnitude greater than the pulmonary EROD and PH activities. The UGT activity was low in lung

microsomes, except for one sample, which glucuronidated 1-OHP between 37 and 69 times faster than the other four samples. The NQO1 measurements showed

Table 1 Inducibility of PAH-metabolizing enzyme activities by a 3-day treatment with individual 'biomarker' PAH compounds and modulators of PAH metabolism in rat lung and liver

Treatment[a]	No. of rats	EROD, pmol/min/mg protein	PH (pyrene hydroxylase), pmol/min/mg protein	N-UGT (1-naphthol), nmol/min/mg protein	1-OHP UGT (1-OH-pyrene), nmol/min/mg protein	E-UGT (entacapone), nmol/min/mg protein	NQO1 (DCPIP), nmol/min/mg protein	GST (CDNB), nmol/min/mg protein
Lung								
Control	8	16 ± 2	8 ± 1	2.60 ± 0.30	0.72 ± 0.11	0.068 ± 0.028	383 ± 23	152 ± 24
Environmental PAHs								
Naphthalene	8	18 ± 3	6 ± 2	2.51 ± 0.14	0.72 ± 0.13	0.071 ± 0.004	575 ± 45***	187 ± 16*
Phenanthrene	4	19 ± 4	9 ± 2	2.83 ± 0.17	0.65 ± 0.09	0.068 ± 0.009	392 ± 89	183 ± 49
Pyrene	8	30 ± 4***	10 ± 2	2.52 ± 0.19	0.67 ± 0.04	0.085 ± 0.006	510 ± 83*	196 ± 29*
Chrysene	4	895 ± 163***	158 ± 31***	3.94 ± 0.18***	1.08 ± 0.04***	0.108 ± 0.004*	$1,350 \pm 309$***	185 ± 38
BaP	4	808 ± 89***	177 ± 17***	4.72 ± 0.38***	1.37 ± 0.15***	0.122 ± 0.011**	$1,680 \pm 232$***	219 ± 3**
Metabolic modulators								
ANF	4	275 ± 24***	89 ± 23***	2.07 ± 0.31	0.71 ± 0.04	0.055 ± 0.014	813 ± 106***	170 ± 11
BNF	4	916 ± 219***	287 ± 69***	5.08 ± 0.58***	1.93 ± 0.16***	0.146 ± 0.006***	$2,921 \pm 722$***	240 ± 62*
MC	4	760 ± 90***	158 ± 12***	3.87 ± 0.50***	1.09 ± 0.08***	0.105 ± 0.013*	$1,343 \pm 187$***	196 ± 18*
BSO	4	14 ± 4	6 ± 1**	2.10 ± 0.09***	0.55 ± 0.05*	0.046 ± 0.010	424 ± 72	147 ± 19
BSO + MC	2	153 ± 19***	165 ± 24***	4.45 ± 0.32***	1.39 ± 0.34***	0.096 ± 0.025	$1,460 \pm 238$***	160 ± 46
EQ	4	53 ± 24*	11 ± 5	2.68 ± 0.28	0.64 ± 0.12	0.062 ± 0.012	601 ± 221*	184 ± 27
EQ + MC	2	112 ± 6***	158 ± 1***	4.47 ± 0.15***	1.29 ± 0.02***	0.120 ± 0.008*	$2,672 \pm 233$***	251 ± 8**
		EROD, nmol/min/mg protein	PH (pyrene hydroxylase), nmol/min/mg protein	N-UGT (1-naphthol), nmol/min/mg protein	1-OHP UGT (1-OH-pyrene), nmol/min/mg protein	E-UGT (entacapone), nmol/min/mg protein	NQO1 (DCPIP), nmol/min/mg protein	GST (CDNB), nmol/min/mg protein
Liver								
Control	8	0.15 ± 0.04	0.09 ± 0.01	9.7 ± 1.8	13.4 ± 1.1	0.39 ± 0.04	352 ± 75	$1,284 \pm 192$
Environmental PAHs								
Naphthalene	8	0.13 ± 0.04	0.08 ± 0.01	10.7 ± 1.2	8.0 ± 0.9***	0.37 ± 0.04	588 ± 125***	$1,510 \pm 251$
Phenanthrene	4	0.61 ± 0.16***	0.17 ± 0.03**	13.2 ± 3.2	10.5 ± 1.7*	0.48 ± 0.10	620 ± 176**	$1,514 \pm 45$*
Pyrene	8	1.12 ± 0.10***	0.17 ± 0.03***	11.2 ± 0.8	8.2 ± 0.6***	0.41 ± 0.04	540 ± 113***	$1,409 \pm 158$
Chrysene	4	30.39 ± 7.17***	1.81 ± 0.38***	21.5 ± 4.7***	17.6 ± 5.3	0.93 ± 0.18***	$1,352 \pm 177$***	$1,759 \pm 230$**
BaP	4	41.88 ± 5.13***	2.48 ± 0.24***	46.5 ± 8.9***	28.7 ± 6.6***	2.49 ± 0.50***	$1,867 \pm 260$***	$2,117 \pm 352$**
Metabolic modulators								
ANF	4	0.86 ± 0.12***	0.36 ± 0.02***	17.4 ± 2.0***	18.1 ± 2.1***	0.70 ± 0.09***	893 ± 82***	$1,632 \pm 251$*
BNF	4	48.59 ± 8.15***	2.25 ± 0.31***	41.6 ± 4.5***	41.8 ± 4.1***	2.54 ± 0.32***	$2,440 \pm 481$***	$2,275 \pm 400$**
MC	4	52.70 ± 9.37***	2.64 ± 0.14***	37.7 ± 3.4***	47.2 ± 4.8***	2.99 ± 0.37***	$2,649 \pm 853$***	$2,325 \pm 428$**
BSO	4	0.08 ± 0.02	0.08 ± 0.02	13.8 ± 1.5***	10.2 ± 0.3***	0.49 ± 0.06**	499 ± 51**	$1,442 \pm 98$
BSO + MC	2	33.40 ± 7.57***	2.81 ± 0.21***	49.7 ± 3.6***	38.4 ± 2.8***	2.83 ± 0.18***	$4,363 \pm 624$***	$3,045 \pm 74$***
EQ	4	0.99 ± 0.47***	0.27 ± 0.04***	23.0 ± 4.8***	13.0 ± 2.1	1.01 ± 0.23***	$1,390 \pm 336$***	$2,953 \pm 431$***
EQ + MC	2	28.34 ± 1.04***	2.82 ± 0.02***	47.7 ± 0.4***	38.6 ± 1.8***	2.51 ± 0.12***	$6,851 \pm 433$***	$4,182 \pm 51$***

Values are the mean ± SD

BaP benzo(a)pyrene, ANF α-naphthoflavone, BNF β-naphthoflavone, MC 3-methylcholanthrene, BSO L-buthionine-[S,R]-sulfoximine, EQ ethoxyquin

*$P < 0.05$ [differs from respective control group (Student's t-test)]

**$P < 0.01$ [differs from respective control group (Student's t-test)]

***$P < 0.001$ [differs from respective control group (Student's t-test)]

[a] Treatment of female Wistar rats, see Sect. 'Methods'

Table 2 Correlation (R^2) matrix for inducible PAH-metabolizing enzyme activities in the rat model. Intrahepatic, intrapulmonary, and intrahepatopulmonary relationships are shown for the activities determined in each of 60 rats (for treatment with single PAHs or model inducers see Table 1)

Enzyme assay	1	2	3	4	5	6	7
Pulmonary	Pulmonary activity						
1. EROD	1	0.90	0.60	0.60	0.46	0.70	0.17
2. PH	0.90	1	0.60	0.69	0.48	0.82	0.20
3. N-UGT	0.60	0.60	1	0.79	0.77	0.69	0.27
4. UGT	0.60	0.69	0.79	1	0.71	0.74	0.13
5. E-UGT	0.46	0.48	0.77	0.71	1	0.56	0.21
6. NQO1	0.70	0.82	0.69	0.74	0.56	1	0.39
7. GST	0.17	0.20	0.27	0.13	0.21	0.39	1
Hepatic	Hepatic activity						
1. EROD	1	0.96	0.75	0.67	0.79	0.72	0.40
2. PH	0.96	1	0.84	0.77	0.87	0.79	0.47
3. N-UGT	0.75	0.84	1	0.81	0.96	0.83	0.65
4. UGT	0.67	0.77	0.81	1	0.88	0.66	0.44
5. E-UGT	0.79	0.87	0.96	0.88	1	0.82	0.62
6. NQO1	0.72	0.79	0.83	0.66	0.82	1	0.72
7. GST	0.40	0.47	0.65	0.44	0.62	0.72	1
Pulmonary	Hepatic activity						
1. EROD	0.82						
2. PH		0.90					
3. N-UGT			0.62				
4. UGT				0.63			
5. E-UGT					0.36		
6. NQO1						0.74	
7. GST							0.15

All R^2 values were significant ($P \leq 0.02$)

that this cytosolic activity was both high and variable (64-fold variability) in human lungs.

Glucuronidation kinetics in rat and human lung

1-Hydroxypyrene glucuronidation kinetics were compared in human and rat lung microsomes. The initial velocities assayed over 1–300 μM 1-OHP concentrations indicated multiphasic enzyme kinetics (Fig. 3). The plots obtained by computing the velocities over 1–10 μM 1-OHP concentration range obeyed hyperbolic kinetics, giving the apparent constants for control and BaP rats: $K_{m1} = 0.8$ μM, $V_{max1} = 0.40$ nmol/min/mg; $K_{m1} = 0.6$ μM, $V_{max1} = 0.58$ nmol/min/mg, respectively (Fig. 3, mid panels). By using the Eadie–Hofstee plot method (Fig. 3, right-side panels), we could resolve two components both in control (K_{m1}, $K_{m2} = 0.8$, 63 μM, V_{max1}, $V_{max2} = 0.39$, 1.84 nmol/min/mg) and BaP-induced rat lungs (K_{m1}, $K_{m2} = 0.6$, 55 μM, V_{max1}, $V_{max2} = 0.57$, 2.51 nmol/min/mg). In human lung, the glucuronidation of 1-OHP seemed to be catalyzed by two low-K_m forms (Fig. 3). The results on UGT kinetics are summarized in Table 4. Note that BaP was a modest inducer of 1-OHP glucuronidation in rat lung, and the intrinsic clearance values (V_{max}/K_m) of the

K_{m1}-form (500 and 984 μl/min/mg) were 6–12 times greater in rat lung microsomes than in human lung microsomes (83 μl/min/mg). The human K_{m2} enzyme (68 μl/min/mg) was a better catalyst than the rat form.

Discussion

Polycyclic aromatic hydrocarbon-metabolizing enzyme activities and their inducibility by individual 'biomarker' PAHs were investigated in the rat to predict effects inducible in human lung by exposure to environmental and industrial PAH mixtures.

In the rat, the efficacy of individual PAHs to enhance pulmonary PH and EROD activity ranged from no effect up to a 22- and 51-fold increase, the order range for the compounds being: naphthalene, phenanthrene < pyrene << chrysene < BaP. As an inducer, BaP proved to be as effective as BNF or MC. Our results and those by Shimada et al. (1992) indicate that the BNF induces EROD activity in rat liver (324-fold versus 678-fold) and lung (57-fold versus 5-fold).

NQO1 activity in the rat showed comparable liver-to-lung tissue levels. Induction by naphthalene, phenanthrene, and pyrene was modest both in rat lung ($\leq$1.5-fold) and liver ($\leq$1.8-fold) compared with a 4.4- to 5.3-fold increase seen after BaP, respectively. The combined treatment with a monofunctional (EQ) and a bifunctional (MC) inducer of NQO1 was anticipated to result in a maximum expression (Nioi and Hayes 2004). This was observed in the liver as a 20-fold increased activity. In the lung, the EQ + MC co-induced activity (7.0-fold) was near to the maximum induced by BNF alone (7.6-fold). The clearly lower inducibility of NQO1 in the lung than that occurring in the liver as noticed with MC may imply enzyme inactivation/target organ toxicity by this lung carcinogen. The order of inducer efficiency in the liver was naphthalene, phenanthrene, pyrene, BSO < ANF << chrysene, EQ < BaP, BNF, MC < BSO + MC < EQ + MC, and in the lung, phenanthrene, BSO < naphthalene, pyrene, EQ < ANF << chrysene, MC < BSO + MC < BaP < EQ + MC, BNF.

The glucuronidation of 1-naphthol ($\leq$2.0-fold) and 1-OHP ($\leq$2.7-fold) was inducible in rat lung as was the glucuronidation of the antiparkinsonian drug entacapone ($\leq$2.1-fold), which is a hUGT1A9 substrate (Elovaara et al. 2004). The rank order of the individual PAH treatments to increase glucuronidation was: naphthalene, phenanthrene, pyrene << chrysene, MC < BaP < BNF. Glucuronidation of PAH phenols yields products excreted in urine and bile (Bock et al. 1998).

Table 3 PAH-metabolizing enzyme activities in human lung

Human lung	Gender and age (years)[a]	EROD, pmol/min/mg microsomal protein	PH, pmol/min/mg microsomal protein	UGT (1-OHP)[b], pmol/min/mg microsomal protein	mEH (NP-oxide), nmol/min/mg	mEH (styrene oxide), nmol/min/mg	NQO1 (cytosol), nmol/min/mg
K1	Male (71)	<0.6	1.65	14	11.51	2.65	2.63
K2	Male (67)	0.8	1.60	8	8.60	1.86	<0.34
K3	Female (69)	<0.6	2.79	15	11.52	2.86	21.85
K12	Female (25)	<0.6	1.16	15	8.90	2.36	2.17
K13	Male (57)	0.6	1.60	552	10.26	2.00	21.72
Mean ± SD (geom. mean)	58 ± 19 (54)	0.6 ± 0.1[c] (0.6)	1.76 ± 0.61 (1.69)	121 ± 241 (27)	10.16 ± 1.39 (10.08)	2.35 ± 0.42 (2.32)	9.74 ± 11.03[c] (3.92)

[a] The samples were from four current non-smokers (K1, K2, K3, K13) and one never-smoker (K12), non-cancerous lung surgery patients with pulmonary emphysema (K1, K2, K3), atelectasis (K12), and granulomatous lesions (K13)

[b] Substrate conc.—136 μM 1-OHP

[c] Calculated by using the detection limit values (0.6 for < 0.6; 0.34 for < 0.34)

Glutathione *S*-transferase alpha, mu, and pi forms catalyze glutathione conjugation to CDNB (Hayes and Pulford 1995). This overall GST activity increased only up to 1.6 times in rat lung. In human lung, this GST activity was increased only by 20% in smokers compared with that in non-smokers (Nakajima et al. 1995). Despite numerous epidemiological lung cancer studies, the etiological role of functional/genetic polymorphism in pulmonary GSTs has remained elusive (Thier et al. 2003; Ye et al. 2006; Vineis 2004).

The hepatic activities of all the other rat enzymes except NQO1 were many times higher than the corresponding pulmonary activities. In control rat liver, the EROD, PH, N-UGT, 1-OHP UGT, E-UGT, and GST activities were 9, 11, 4, 19, 6, and 8 times greater, respectively, than the lung activity. In B*a*P-induced liver, these activities were 52, 14, 10, 20, 21, and 10 times greater, respectively, than the lung activity. The hepatic and pulmonary induction profiles by individual PAHs were similar, whereas the efficiency differed widely: control ≤ naphthalene < phenanthrene, pyrene << chrysene < B*a*P. A similar rank order was reported for the inducibility of phenanthrene metabolism in rat liver microsomes by single PAHs (Jacob et al. 1982). The response by B*a*P was in the same range as the induction levels found in BNF- or MC-treated rats. In conclusion, the inducing efficiency by naphthalene, phenanthrene, and pyrene is weak compared to the efficiency by the two well-known bay-region PAH carcinogens: chrysene and B*a*P.

Enzyme kinetics of 1-OHP UGT in rat lung microsomes suggested catalysis by at least two inducible forms in the low-K_m range 0.6–63 μM. The apparent intrinsic clearance by these two components was 1.6–1.9 times lower in control rats than in the B*a*P-induced rats. Also, in human lung microsomes, 1-OHP was glucuronidated by two forms in the K_m range 6–11 μM. We were able to evaluate the glucuronidation kinetics in one individual (K13) who had an exceptionally high pulmonary enzyme activity (552 pmol/min/mg). In the other four subjects, the UGT activity was too low (8–15 pmol/min/mg) to permit an evaluation of enzyme kinetics. Our results support the current understanding that the UGT1A6 and 1A9 are two low-K_m enzymes with a constitutive and inducible expression in human liver and lung, catalyzing the glucuronidation and elimination of PAH phenols (Bock et al. 1998; Luukkanen et al. 2001).

The enzyme activities in human lung were many times lower than the average activities in control rats, this trend being observed for EROD (20–27-fold), PH (5–7-fold), 1-OHP UGT (6–380-fold), and NQO1 (17–1,126-fold). Human mEH activity was 7–9 times
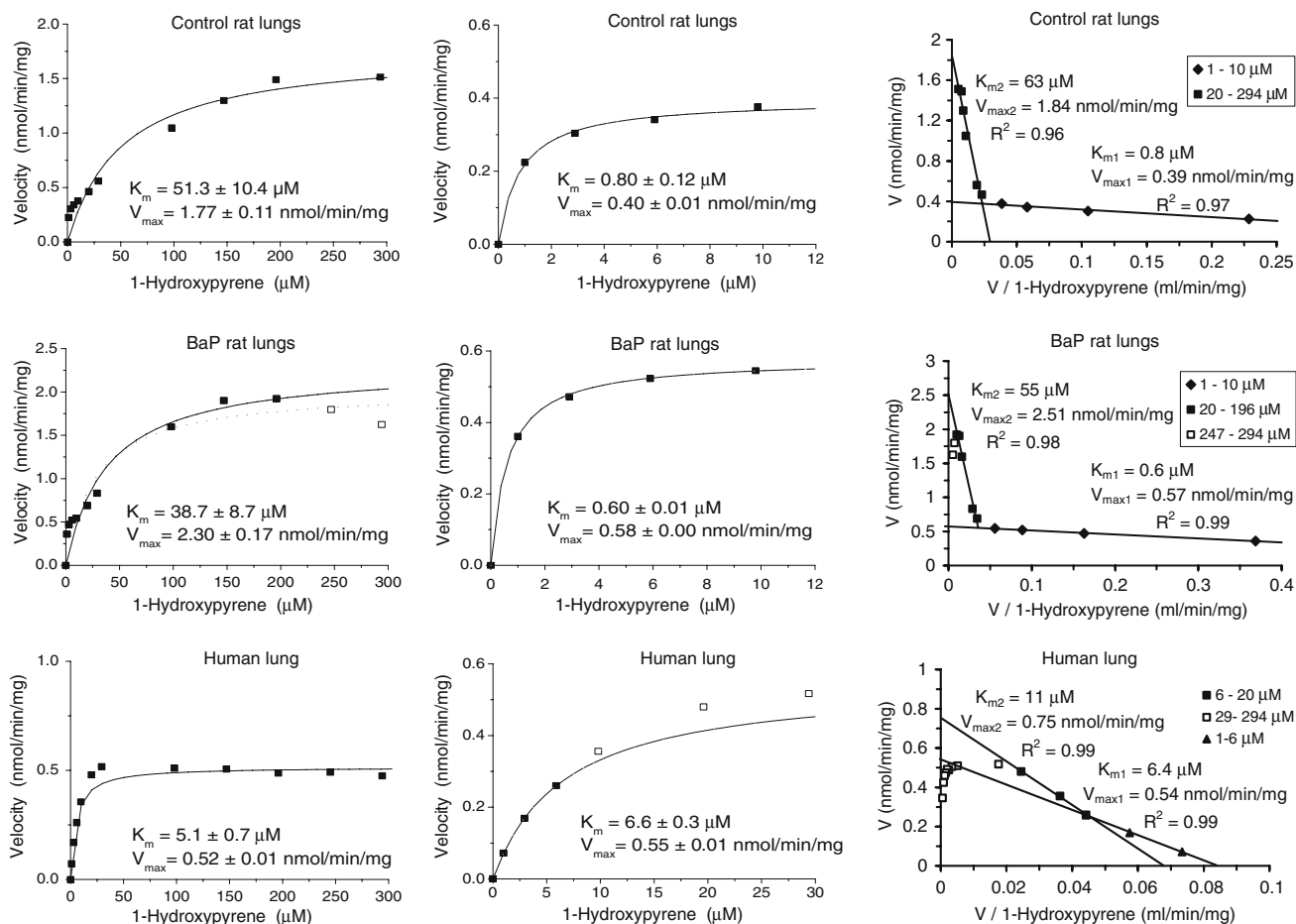
Fig. 3 Enzyme kinetics of 1-OHP UGT in pooled rat lung microsomes of control and BaP-treated groups, and the enzyme kinetics in human lung microsomes from a non-smoking individual (K13) (*bottom line panels*). The apparent K_m and V_{max} values shown in the panels to the *left* and in the *middle* were calculated by the Michaelis–Menten equation. The panels on the *right side* show the K_m and V_{max} values obtained by the Eadie–Hofstee plot method

Table 4 Enzyme kinetics of 1-OHP UGT in rat and human lung

Lung microsomes	K_{m1}, μM	V_{max1}, nmol/min/mg	V_{max1}/K_{m1}, μl/min/mg	K_{m2}, μM	V_{max2}, nmol/min/mg	V_{max2}/K_{m2}, μl/min/mg
Control rat	0.8	0.40	500	63	1.84	29
BaP rat	0.6	0.58	967	55	2.51	46
Human K13	6.6	0.55	83	11	0.75	68

higher than the activity in control rat lung (1.27 ± 0.16 nmol/min/mg, $n = 6$, 0.35 mM NP-oxide; E. Elovaara, unpublished results). Since the lung samples came from non-smoking patients, the CYP1A1 marker activities were low. The EROD values were 80–100 times lower than the average lung activity in cigarette smokers (62 ± 52 pmol/min/mg, $n = 33$) (Anttila et al. 2001). Also, the PH activity was lower than that in most smokers (Elovaara et al. 1997; Vainio et al. 1995). No marked pulmonary induction of AhR-regulated enzymes could be inferred from the EROD and PH results.

Although humans are exposed to airborne PAH mixtures, the estimated pulmonary uptake of potent inducers such as BaP seems frequently to be too low to cause a maximum CYP1A1 induction in human lung (Gundel et al. 2000). CYP1A1 induction levels may thus critically depend on the total PAH uptake, which in turn may vary from one individual to another due to co-exposure of many PAH sources attributable to life style, medication, environment, or occupation (Bouchardt and Viau 1999). Such differences in the internal exposure may explain some of the large variations (200-fold) observed in EROD activity among lung cancer

patients (Anttila et al. 2000). The constitutive expression of CYP1A1 is very low in human lung (Hukkanen et al. 2002; Kim et al. 2004a). It is therefore evident that CYP1A1 catalyzed bioactivation of PAH procarcinogens to DNA reactive products (Penning et al. 1999; Shimada and Fujii-Kuriyama 2004) depend on its inducible expression in human lung (Vineis 2004).

The activity of NQO1 in human lung was about 17–1,000 times lower than the basal levels in rat lung. The human activities varied by up to 64-fold in subjects whose PH and EROD levels were too low to indicate CYP1A1 induction. This large interindividual variation is probably explained by genetic polymorphisms. The associations between low-activity/low-inducibility NQO1 genotypes and human lung cancer risk have been reviewed by Ross and Siegel (2004). NQO1 is a PAH-inducible enzyme in human lung (Schlager and Powis 1990; Jaiswal 2000). It is induced even by naphthalene, which is transformed into reactive and redox active o-quinones, inducing NQO1 via electrophilic and oxidative stress (Ross and Siegel 2004). NQO1 protects against chemical toxicity and neoplasia by catalyzing two-electron reduction of highly reactive PAH quinones (Joseph and Jaiswal 1994; Penning et al. 1999). The anticarcinogenic properties of NQO1 are attributable to its role in antioxidant defense and stabilization of the tumor suppressor protein p53 (Asher et al. 2004).

The specific activity of mEH toward styrene oxide and NP-oxide was over 1,000 times greater than the corresponding PH and EROD activities in human lung. As shown previously (Nakajima et al. 1995), the mean pulmonary activity of mEH (NP-oxide) in smokers is about 5,000 times and, in non-smokers, about 80,000 times greater than the corresponding activity of BaP hydroxylation. Despite a 13-fold increase in BaP hydroxylation, no co-induction of mEH was detected in the smokers' lungs. mEH catalyzes the formation of dihydrodiols which are either activated by oxygenation to diol epoxides (ultimate carcinogens) or converted to excretable products, usually, by glucuronidation or sulfation (Gelboin 1980; Conney 1982). The results of the many epidemiological studies into the associations between polymorphic mEH genotypes (low/high activity) and lung cancer risk are conflicting (Kiyohara et al. 2002). Interindividual differences in this highly expressed enzyme may have little impact on the PAH-metabolizing capacity of mEH in human lung.

In human lung, the glucuronidation rate could be probed only with 1-OHP. This is a sensitive substrate for detecting hUGT1A6/hUGT1A9-related activity (Luukkanen et al. 2001; Paakki et al. 2000). Several recombinant forms of human UGT1A family (1A1, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10) are able to glucuroni-

date 1-OHP (Luukkanen et al. 2005). The UGT activity in human lung microsomes (0.008–0.552 nmol/min/mg) was 380 to 6 times lower than the activity in control rats (3.03 ± 0.33 nmol/min/mg). UGT levels in human lung varied widely, both in this (69-fold) and our previous studies (700-fold; 0.0003–0.216 nmol/min/mg) (Vainio et al. 1995). Evidence is emerging that UGT polymorphisms may play a role in the susceptibility to smoking-related cancer (Jinno et al. 2003; Wells et al. 2004).

In conclusion, the EROD, PH, NQO1, mEH, 1-OHP UGT measurements were limited to five lung samples from non-smoking non-cancer patients. Nevertheless, extensive interindividual differences could already be found among the human activities of UGT and NQO1, and these were relatively greater than the experimental (inducible/constitutive) variation in the rat. The activities attributable to pyrene oxidation (CYP1A1) and glucuronidation (UGT1A6/1A9) were very low in human lung when compared with that of mEH and NQO1. It appears that the CYP1A1 expression is dictated by the degree of enzyme induction while the large variability in expression of UGT1A and NQO1 is predominantly dictated by one's genetic constitution. If the PAH-metabolizing activities could be tested in lung samples from individuals with high occupational PAH exposure (via lung and skin), and compared with our results, we might well find up to 100 times higher EROD and PH activities, higher and more variable NQO1 and UGT activities, and least changes in mEH activity. Since the 2–4-ring PAHs (major constituents in PAH mixtures) were poor enzyme inducers compared with BaP (minor constituent), it appears that enzyme induction in human lung is a response evoked mainly by exposure to carcinogenic BaP-type hydrocarbons. Gene–environmental interactions which magnify polymorphic variability in pulmonary bioactivation/detoxification capacity probably play a significant role in individual susceptibility to (or protection against) chemically induced lung cancer. Exposure to PAH mixtures with a high content of BaP-type hydrocarbons appears to confer a potentially higher health risk than PAH mixtures with a low content of procarcinogens.

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