Available online at www.sciencedirect.com
ScienceDirect
Toxicology 231 (2007) 68–80
TOXICOLOGY
www.elsevier.com/locate/toxicol

Evaluation of the precision-cut liver and lung slice systems for the study of induction of CYP1, epoxide hydrolase and glutathione *S*-transferase activities

Daphnee S. Pushparajah, Meera Umachandran, Kathryn E. Plant, Nick Plant, Costas Ioannides *

Molecular Toxicology Group, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK

Received 12 October 2006; received in revised form 20 November 2006; accepted 21 November 2006
Available online 26 November 2006

Abstract

The principal objective was to ascertain whether precision-cut tissue slices can be used to evaluate the potential of chemicals to induce CYP1, epoxide hydrolase and glutathione *S*-transferase activities, all being important enzymes involved in the metabolism of polycyclic aromatic hydrocarbons. Precision-cut rat liver and lung slices were incubated with a range of benzo[a]pyrene concentrations for various time periods. A rise in the *O*-deethylation of ethoxyresorufin was seen in both liver and lung slices exposed to benzo[a]pyrene, which was accompanied by increased CYP1A apoprotein levels. Pulmonary CYP1B1 apoprotein levels and hepatic mRNA levels were similarly enhanced. Elevated epoxide hydrolase and glutathione *S*-transferase activities were also observed in liver slices following incubation for 24 h; similarly, a rise in apoprotein levels of both enzymes was evident, peak levels occurring at the same time point. When mRNA levels were monitored, a rise in the levels of both enzymes was seen as early as 4 h after incubation, but maximum levels were attained at 24 h. In lung slices, induction of epoxide hydrolase by benzo[a]pyrene was observed after a 24-h incubation, and at a concentration of 1 μM; a rise in apoprotein levels was seen at this time point. Glutathione *S*-transferase activity was not inducible in lung slices by benzo[a]pyrene but a modest increase was observed in hepatic slices. Collectively, these studies confirmed CYP1A induction in rat liver slices and established that CYP1B1 expression, and epoxide hydrolase and glutathione *S*-transferase activities are inducible in precision-cut tissue slices.

Keywords: Precision-cut tissue slices; CYP1; Epoxide hydrolase; Glutahtione *S*-transferase; Benzo[a]pyrene

1. Introduction

The toxicity and carcinogenicity of many chemicals, both naturally occurring and anthropogenic, are inextricably linked to their metabolism to reactive, electrophilic intermediates that interact covalently with vital cellular macromolecules leading to mutations and toxicity. For example, polycyclic aromatic hydrocarbons, a major class of environmental and dietary carcinogens, are metabolised by the CYP1 family of cytochromes P450 to form epoxides, which are detoxicated through the action of epoxide hydrolase and glutathione *S*-transferase; the former enzyme converts the epoxides to *trans*-dihydrodiols that are further oxidised by the CYP1 family to dihydrodiol-epoxides, the ultimate car-

* Corresponding author. Tel.: +44 1483 689709; fax: +44 1483 686401.
E-mail address: c.ioannides@surrey.ac.uk (C. Ioannides).

0300-483X/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2006.11.063

cinogens, whereas the latter catalyses the conjugation of epoxides with reduced glutathione, the conjugate being usually further metabolically processed and excreted as the mercapturate (Pelkonen and Nebert, 1982; Ioannides and Parke, 1990). Indeed, epoxides have been implicated in the toxicity/carcinogenicity of many other structurally diverse chemicals including mycotoxins such as aflatoxin B_1 (Guengerich et al., 1998), and halogenated aliphatic compounds such as vinyl chloride (Guengerich, 2003); their formation is most frequently catalysed by cytochromes P450.

Precision-cut tissue slices have been prepared from a number of animal species, including human, and are increasingly being used in toxicology (De Kanter et al., 1999; Lerche-Langrand and Toutain, 2000). They have been employed successfully to unravel the metabolic pathways of xenobiotics (Ekins, 1996), evaluate the toxicity of chemicals (Miller et al., 1993; Price et al., 1996; van de Bovenkamp et al., 2005), study the regulation of enzyme systems such as the cytochromes P450 (Meredith et al., 2003), investigate the hepatic uptake of drugs (Onderwater et al., 2005), evaluate the genotoxic and antigenotoxic potential of chemicals (Baumann et al., 1996; Lake et al., 1999), activation of signalling pathways (Pfaff et al., 2005) and to determine chemically induced apoptosis (Moronvalle-Halley et al., 2005).

It has already been established that cytochrome P450 enzymes can be induced in hepatic, intestinal and pulmonary rat slices following incubation with appropriate inducing agents (Lake et al., 1993, 1996, 2003; Müller et al., 2000; Martignoni et al., 2004; Harrigan et al., 2006). Similarly, induction of this enzyme system takes place in precision-cut human hepatic slices (Edwards et al., 2003). In contrast, the inducibility of other xenobiotic-metabolising enzymes in precision-cut tissue slices has not been established. In this paper we document the inducibility of CYP1A1, CYP1B1, microsomal epoxide hydrolase and cytosolic glutathione *S*-transferase, three key enzyme systems in the metabolism of polycyclic aromatic hydrocarbons, in liver and lung slices, monitored at the activity, apoprotein and mRNA levels, following incubation with benzo[a]pyrene, the prototype polycyclic aromatic hydrocarbon.

2. Materials and methods

Benzo[a]pyrene 4,5-epoxide and benzo[a]pyrene 4,5-diol (Mid-West Research Institute, Kansas, USA), rat genomic DNA (Novagen, Wisconsin, USA), 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (Fluka, Buchs SG, Switzerland), Superscript II (Invitrogen, Paisley, UK), RNase-free DNase (Promega, Wisconsin, USA), NADPH, benzo[a]pyrene, ethoxyresorufin, resorufin, 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB), peroxidase-linked anti-rabbit, anti-goat and anti-sheep antibodies (Sigma Co. Ltd., Poole, Dorset, UK), Qiagen RNeasy Mini kits (Crawley, West Sussex, UK), Absolute™ QPCR Mix (Abgene, Epsom, Surrey, UK), and Earle's balanced salt solution (EBSS), foetal calf serum, gentamycin and RPMI with L-glutamine culture medium (Invitrogen, Paisley, Scotland) were all purchased. Twelve-well plates were obtained from Bibby Sterilin (Helena Biosciences, Sunderland, UK). Rat anti-CYP1A1 and anti-CYP1B1 antibodies were obtained from BD Biochemicals (Oxford, UK), and antibody to the Ya subunit of the α-family of glutathione *S*-transferases from Oxford Biomedical Research (Oxford, USA), whereas antibody to microsomal epoxide hydrolase was a kind gift from Professor M. Arand (Herrero et al., 1997).

Male Wistar albino rats (200 g) were obtained from B&K Universal Ltd. (Hull, East Yorkshire, UK). The animals were housed at 22 ± 2 °C, 30–40% relative humidity in an alternating 12-h light:12-h dark cycle with light onset at 07.00 h. Rats were killed by cervical dislocation, liver was immediately excised, and liver slices (250 μm) were prepared from 8 mm cylindrical cores using a Krumdieck tissue slicer (Alabama Research and Development Corporation, Munsford, AL, USA) as previously described (Hashemi et al., 1999a). The multiwell plate procedure, using 12-well culture plates, was used to culture the slices. The culture medium was essentially that described by Lake et al. (1993) and comprised RPMI 1640 Glutamax II containing foetal calf serum (5%), L-methionine (0.5 mM), insulin (1 μM), gentamycin (50 μg/mL) and hydrocortisone 21-hemisuccinate (0.1 mM). One slice was placed in each well, in 1.5 ml of culture medium. Slices were incubated under sterile conditions on a reciprocating plate shaker housed in a humidified incubator, at a temperature of 37 °C and under an atmosphere of 95% air/5% CO_2. The slices were initially pre-incubated for 30 min in order to slough off any dead cells due to slicing. For the production of lung slices, animals were killed by an overdose of sodium pentobarbital, and lungs were perfused intratracheally with agarose (0.75%, v/w) at 37 °C. Agar was allowed to solidify, and lung slices (600 μm) were prepared from cylindrical cores (8 mm) as described for the liver (Umachandran et al., 2004). A pre-incubation of 60 min was carried out. For incubations exceeding 24 h, slices were placed in fresh medium every 24 h. Three different slice pools, comprising 4–10 slices, were used per time point.

Following incubation, slices were removed from the medium, homogenised, and microsomal and cytosolic fractions were prepared. Epoxide hydrolase was determined in the microsomal fraction using benzo[a]pyrene 4,5-epoxide as substrate (Dansette et al., 1979), whereas glutathione *S*-transferase using CDNB, DCNB (Habig et al., 1974) and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (Ricci et al., 1994) as substrates was determined in the cytosol. The *O*-deethylation of ethoxyresorufin (Burke and Mayer, 1974) was determined in the hepatic microsomes, but in the lung studies activity was determined in the post-mitochondrial fraction (S9); in this instance the

incubation system was supplemented with dicoumarol (8 μM). Protein was determined in all tissue fractions (Bradford, 1976). Finally, in order to determine apoprotein levels, hepatic microsomal proteins were resolved by electrophoresis and incubated with the primary antibody and the corresponding peroxidase-linked secondary antibody. Immunoblots were quantitated by densitometry using the GeneTool software (Syngene Corporation, Cambridge, UK). In preliminary studies, a linear response was established with protein levels ranging 50–400 μg of protein (results not shown).

Two slices were used for total RNA extraction, and for each sample triplicates were carried out. RNA was extracted using the Qiagen RNeasy Mini kit and was quantified using a Nanodrop spectrophotometer. Total RNA was treated with RNase-free DNase to remove genomic contamination. Reverse transcription was primed with random hexamers and carried out by Superscript II according to the manufacturer's instructions. To ensure that DNase treated samples were free from genomic contamination, an RT-control was carried out for every RNA sample. cDNA generated from 50 ng was amplified using Absolute™ QPCR Mix with 400 nM primers and 100 nM fluorogenic probe in a total reaction volume of 25 μl. Q-PCR reactions were run on the ABI7000 SDS instrument (Applied Biosystems, Warrington, UK) and quantitation was carried out using the ABI proprietary software against a standard curve generated from rat genomic DNA.

For the quantitative reverse transcription-polymerase chain reaction, the primers and TAMRA/FAM dual labelled probes (Table 1) were designed using the Primer Express software (Applied Biosystems) and purchased from MWG, Ebersberg, Germany. Each primer and probe set was designed to amplify sequences within a single exon, so that genomic DNA could be used as a standard.

Statistical evaluation was carried out using the Student's *t*-test.

3. Results

Ethoxyresorufin *O*-deethylase activity decayed with incubation time in liver slices but was still detectable after 48 h (Fig. 1A). Exposure of the slices to benzo[a]pyrene resulted in a marked elevation of the activity, which was most pronounced after a 24-h incubation. The rise in activity was paralleled by an increase in CYP1A apoprotein levels (Fig. 1B). At the mRNA level a significant increase was evident after 4 h, but was far more pronounced on longer incubation (Fig. 1C). Induction of ethoxyresorufin *O*-deethylase activity, following 24-h exposure, was concentration-dependent up to a concentration of 10 μM, but declined at higher concentrations (Fig. 2A). The same picture emerged when CYP1A1 apoprotein levels were monitored (Fig. 2B). A rise in CYP1A1 mRNA levels was observed at all concentrations of benzo[a]pyrene employed, but maxi-

Table 1
Taq Man® primers and probes for rat CYP1A1, CYP1A2, glutathione *S*-transferase and epoxide hydrolase

Gene	Accession number	Location	5′ Primer	3′ Primer	Probe
CYP1A1	NM_012540	97–122	GCCTTCACATCAGCCACAGA	TTGTGACTCTAACCACCCAGAATC	TGGCCGTCACCACATTCTGCCTT
CYP1B1	NM_012940	2696–2775	TTCAGCTGTTCAAACGAAGCA	TCCCAAAGTTGAAGCTTACGTTA	CGAGTTATGAGGGAGAAAAAGGTTTGCCA
Epoxide hydrolase	NM_012844	461–537	TCTATGAGTTTTAAAGATCATCCCACTA	TTCAAACACGTGCTCGTCACT	TGACTGACCCCAAGTCCCACGGTC
Glutathione *S*-transferase1/2	NM_017013	489–584	CCATGGCCAAGACTACCTTGTAG	AGGCTGGCATCAAACTCTTCA	CCGGGTAGACATCCACCTGCTGGAAC

The location is relative to position one of the accession number. Probes were labelled with 5′ TAMRA and 3′ FAM.

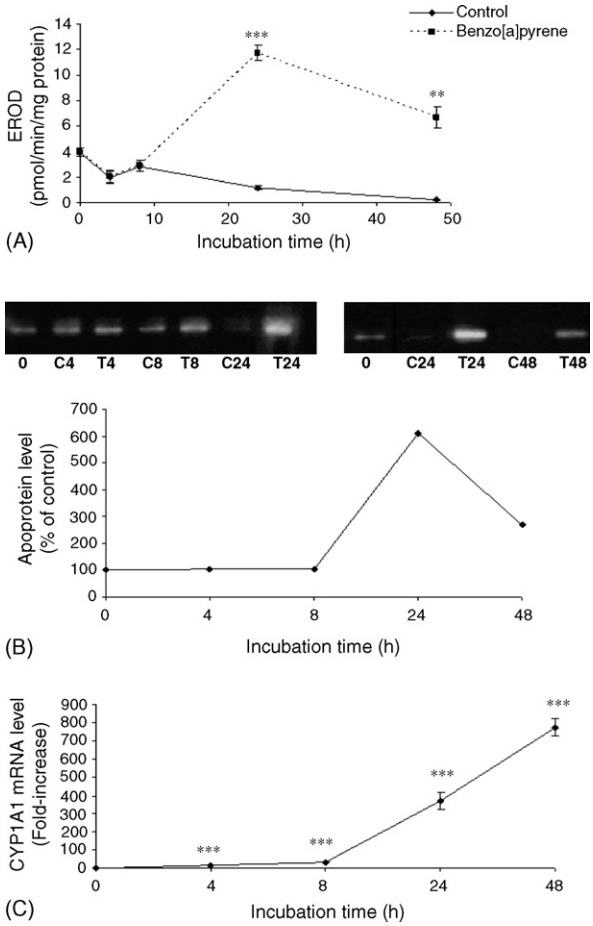

Fig. 1. Time-dependent induction of CYP1A1 by benzo[a]pyrene in rat precision-cut liver slices. Precision-cut liver slices were incubated in the presence and absence of benzo[a]pyrene (10 μM) for various periods of time up to 48 h. At the end of each time period, slices were removed from the media and mRNA or microsomes isolated: (A) ethoxyresorufin *O*-deethylase activity (EROD); (B) apoprotein levels in pooled slices probed with antibodies to rat CYP1A1 with each lane loaded with 5 μg protein, where C refers to control and T to benzo[a]pyrene-treated slices, and number denotes period of incubation in hours; (C) fold-increase in mRNA levels compared to controls where slices were incubated with the corresponding volume of DMSO, the vehicle. Where appropriate, results are expressed as mean ± S.D. of triplicate pools of slices. $^{**}P<0.01$; $^{***}P<0.001$.

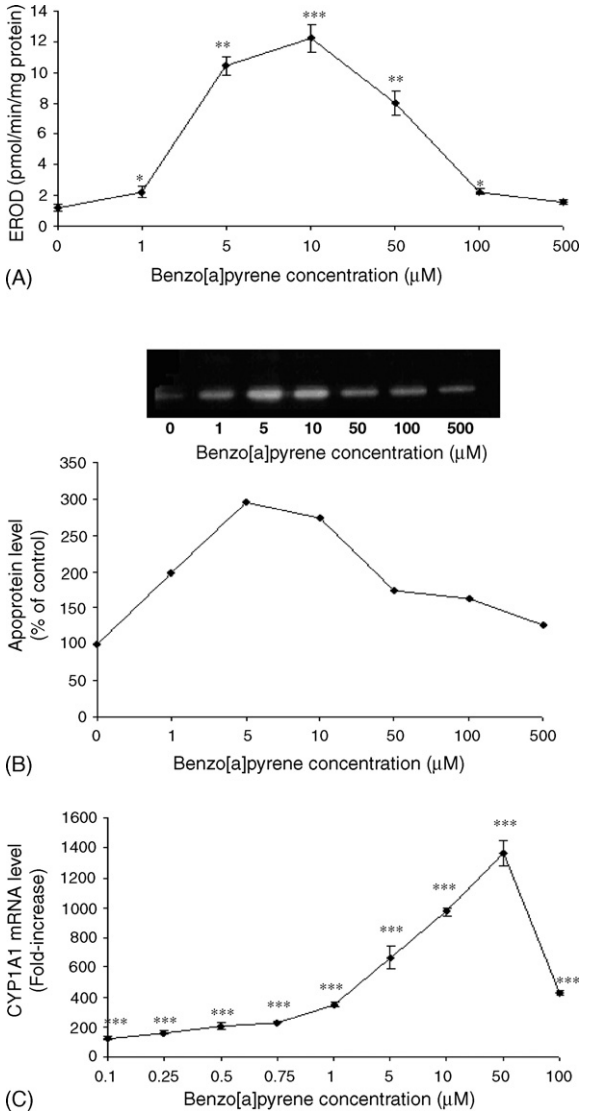

Fig. 2. Concentration-dependent induction of CYP1A1 by benzo[a]pyrene in rat precision-cut liver slices. Precision-cut liver slices were incubated in the presence of a range of benzo[a]pyrene concentrations (0–500 μM) for 24 h. At the end of the incubation period, slices were removed from the media and mRNA or microsomes isolated: (A) ethoxyresorufin *O*-deethylase (EROD); (B) apoprotein levels in pooled slices probed with antibodies to rat CYP1A1 with each lane being loaded with 5 μg protein; (C) fold-increase in mRNA levels compared to controls where slices were incubated with the corresponding volume of DMSO, the vehicle. Where appropriate, results are expressed as mean ± S.D. of triplicate pools of slices. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

mum was attained at the 50 μM concentration; a marked decline was noted at 100 μM, the highest dose investigated (Fig. 2C).

When lung slices were exposed to benzo[a]pyrene (1 μM), a rise in ethoxyresorufin *O*-deethylase activity was once again observed, but the effect was most pronounced after a 48-h incubation (Fig. 3A). Western blot analysis concurred in that CYP1A1 apoprotein levels were elevated after exposure of the lung slices to benzo[a]pyrene, and maximum rise was attained after a 48-h incubation (Fig. 3B). The effect of benzo[a]pyrene on ethoxyresorufin *O*-deethylase activity was concentration dependent, with a maximum being attained at the 1 μM concentration (Fig. 3C), and this was accompanied by a parallel rise in CYP1A1 apoprotein levels (Fig. 3D).

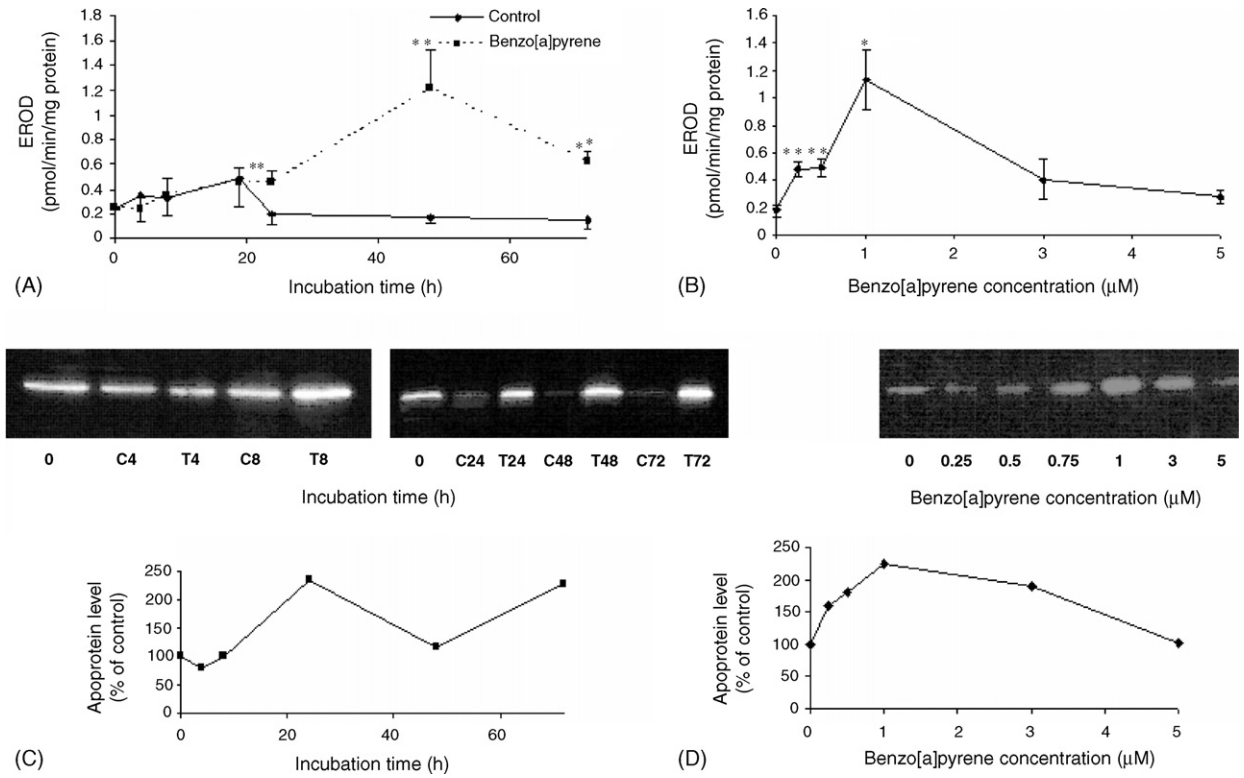

Fig. 3. Time- and concentration-dependent induction of CYP1A1 by benzo[a]pyrene in rat precision-cut lung slices. Precision-cut lung slices were incubated in the presence and absence of benzo[a]pyrene (1 μM) for various periods of time up to 48 h, or for 48 h in the presence of a range of concentrations (0–5 μM) of benzo[a]pyrene. At the end of each incubation, slices were removed from the media and microsomes isolated: (A) ethoxyresorufin *O*-deethylase activity (EROD); (B) apoprotein levels in pooled slices probed with antibodies to rat CYP1A1, where C refers to control and T to benzo[a]pyrene-treated slices, and number denotes period of incubation in hours. Lanes were loaded with 200 (control) or 100 (benzo[a]pyrene-treated) μg of protein; (C) ethoxyresorufin *O*-deethylase activity; (D) apoprotein levels in pooled slices probed with antibodies to rat CYP1A1, where the lane with control sample was loaded with 70 and lanes with benzo[a]pyrene-treated samples with 35 μg of protein. Where appropriate, results are expressed as mean ± S.D. of triplicate pools of slices. Plots of apoprotein levels have been corrected for protein. $^{*}P < 0.05$; $^{**}P < 0.01$.

Incubation of liver slices with benzo[a]pyrene led to an increase of CYP1B1 mRNA levels, which was evident as early as 4 h after incubation, but significantly larger rises were achieved on longer incubations (Fig. 4A). Following incubation of slices with a range of benzo[a]pyrene concentrations, elevated mRNA levels were noted even at the lowest concentration studied (0.1 μM), but a marked rise was observed at concentrations greater than 1 μM (Fig. 4B). In lung slices, CYP1B1 expression was monitored at the apoprotein level. Treatment of lung slices with benzo[a]pyrene (1 μM) doubled CYP1B1 apoprotein levels after a 24-h incubation (Fig. 4C). When the slices were exposed to different concentrations of benzo[a]pyrene, maximum increase was observed at the 1 μM concentration (Fig. 4D).

Epoxide hydrolase activity in rat liver slices declined with time of incubation, with about half of the original activity remaining after 48 h. When slices were incubated with benzo[a]pyrene (10 μM), a statistically significant, but modest, rise in activity was observed after a 24-h incubation (Fig. 5A). Immunoblot analysis showed a similar increase in apoprotein levels occurring after a 24-h incubation (Fig. 5B). At the mRNA level, a significant rise was seen as early as 4 h after incubation, but maximum increase was evident when the slices were exposed to benzo[a]pyrene for 24 h (Fig. 5C). When rat liver slices were incubated with a range of concentrations of benzo[a]pyrene, a statistically significant increase in epoxide hydrolase activity was evident only at the 10 μM concentration, while at higher concentrations activity gradually declined to control levels (Fig. 6A). Similarly, there was a 50% rise in apoprotein levels following exposure to 5–10 μM benzo[a]pyrene, but at higher concentrations the levels declined gradually to control values (Fig. 6B). When mRNA levels

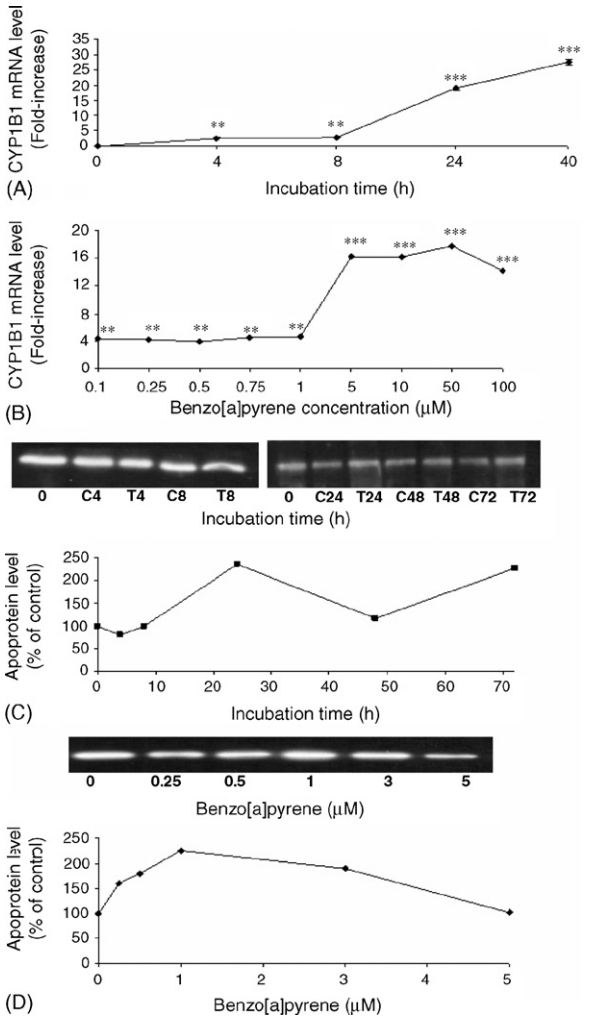

Fig. 4. Time- and concentration-dependent increase in CYP1B1 expression by benzo[a]pyrene in rat precision-cut liver and lung slices. Precision-cut liver and lung slices were incubated in the presence and absence of benzo[a]pyrene, at a concentration of 10 and 1 μM, respectively, for various periods of time up to 48 h, or for 48 h in the presence of a range of concentrations of benzo[a]pyrene. At the end of each incubation, slices were removed from the media and microsomes or mRNA isolated: (A) fold-increase in mRNA levels compared to controls where liver slices were incubated with the corresponding volume of DMSO, the vehicle; (B) fold-increase in mRNA levels compared to controls where liver slices were incubated with the corresponding volume of DMSO; (C) apoprotein levels in pooled lung slices probed with antibodies to rat CYP1B1, where C refers to control and T to benzo[a]pyrene-treated slices, and number denotes period of incubation in hours. In the immunoblot to the left, all lanes were loaded with 200 μg of protein; in the immunoblot to the right, control lane was loaded with 63 and benzo[a]pyrene-treated with 126 μg of protein; (D) apoprotein levels in pooled slices probed with antibodies to rat CYP1B1, where the lane with control sample was loaded with 234 and lanes with benzo[a]pyrene-treated samples with 117 μg of protein. Where appropriate, results are expressed as mean ± S.D. of triplicate pools of slices. Plots of apoprotein levels have been corrected for protein. $^{**}P<0.01$; $^{***}P<0.001$.

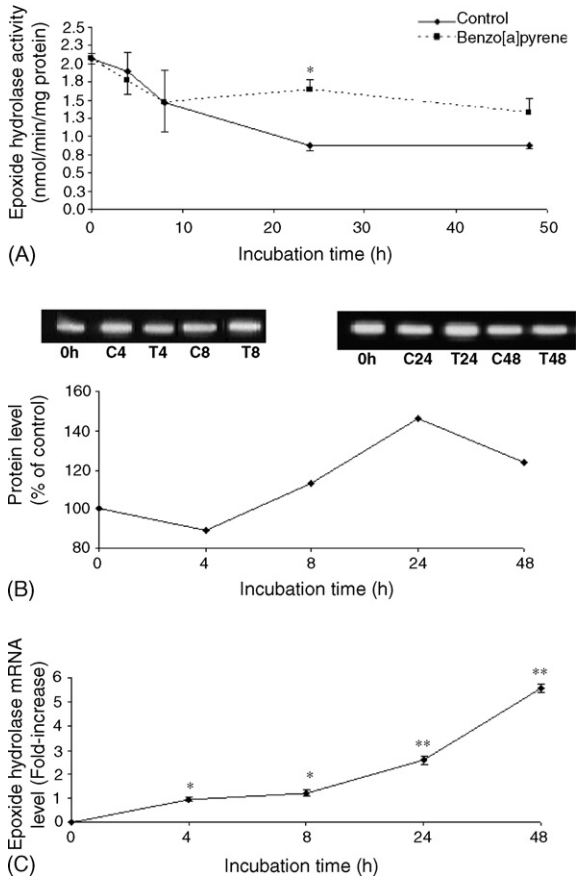

Fig. 5. Time-dependent induction of microsomal epoxide hydrolase by benzo[a]pyrene in rat precision-cut liver slices. Precision-cut liver slices were incubated in the presence and absence of benzo[a]pyrene (10 μM) for various periods of time up to 48 h. At the end of each time period, slices were removed and mRNA or microsomes isolated: (A) epoxide hydrolase activity assessed using benzo[a]pyrene 4,5-oxide as substrate; (B) apoprotein levels in pooled slices probed with antibodies to epoxide hydrolase with all lanes being loaded with 5 μg of protein, where C refers to control and T to benzo[a]pyrene-treated slices, and number denotes period of incubation in hours; (C) fold-increase in mRNA levels compared to controls where slices were incubated with the corresponding volume of DMSO, the vehicle. Where appropriate, results are expressed as mean ± S.D. of triplicate pools of slices. $P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

were monitored, a concentration-dependent increase was revealed; interestingly, although transcript levels peaked at 10 μM, in line with activity and apoprotein levels, mRNA levels did not change at higher concentrations, with the transcript level remaining constant (Fig. 6C).

When similar studies were performed in rat lung slices, a statistically significant increase in epoxide hydrolase activity was attained once again after a 24-h incubation (Fig. 7A), and Western blot analysis concords with these observations (Fig. 7B). Epoxide hydrolase activity was lower in lung slices compared with liver,

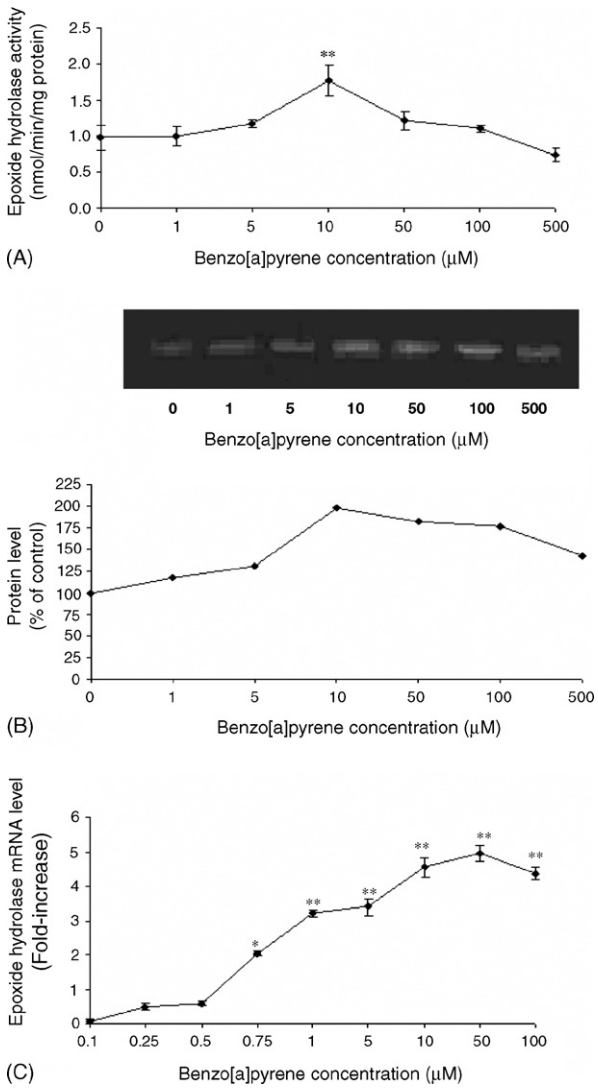

Fig. 6. Concentration-dependent induction of microsomal epoxide hydrolase by benzo[a]pyrene in rat precision-cut liver slices. Precision-cut liver slices were incubated in the presence of a range of benzo[a]pyrene concentrations (0–500 μM) for 24 h. At the end of the incubation period, slices were removed and mRNA or microsomes isolated: (A) epoxide hydrolase activity assessed using benzo[a]pyrene 4,5-oxide as substrate; (B) apoprotein levels in pooled slices probed with antibodies to epoxide hydrolase with all lanes being loaded with 5 μg of protein; (C) fold-increase in mRNA levels compared to controls where slices were incubated with the corresponding volume of DMSO, the vehicle. Where appropriate, results are expressed as mean ± S.D. of triplicate pools of slices. $^{**}P<0.01$; $^{***}P<0.001$.

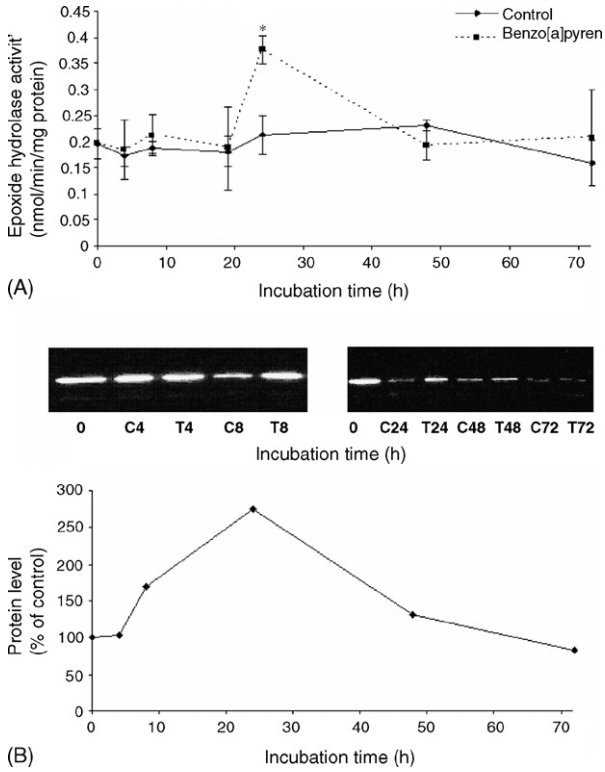

Fig. 7. Time-dependent induction of microsomal epoxide hydrolase by benzo[a]pyrene in rat precision-cut lung slices. Precision-cut lung slices were incubated in the presence and absence of benzo[a]pyrene (1 μM) for various periods of time up to 72 h. At the end of each time period, slices were removed and microsomes isolated: (A) epoxide hydrolase activity assessed using benzo[a]pyrene 4,5-oxide as substrate; (B) apoprotein levels in pooled slices probed with antibodies to epoxide hydrolase, where C refers to control and T to benzo[a]pyrene-treated slices, and number denotes period of incubation in hours. Into each well 62 μg (control) or 31 μg (benzo[a]pyrene-treated) of protein was loaded. Where appropriate, results are expressed as mean ± S.D. of triplicate pools of slices. Plot of apoprotein levels has been corrected for protein. $^{*}P<0.05$.

but when lung slices were incubated with a range of benzo[a]pyrene concentrations, a statistically significant increase in activity was observed at a concentration of 1 μM, and then declined at higher concentrations to below control values (Fig. 8A). Western blot analysis similarly revealed a rise in apoprotein levels, maximum being reached at 1 μM of benzo[a]pyrene, and then levels declined at the higher concentrations (Fig. 8B).

Hepatic glutathione *S*-transferase activity in liver slices was monitored using three substrates, namely CDNB, DCNB and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole. When the former two were used, exposure of slices to benzo[a]pyrene for various times up to 48 h did not modulate activity significantly (results not shown). When 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole served as the substrate, treatment with benzo[a]pyrene led to statistically significant elevation in activity after 24 h of incubation (Fig. 9A). Immunoblot analysis employing antibodies to the Ya subunit recognised a single band, the levels of which were moderately elevated in a concentration-dependent manner, with maximum

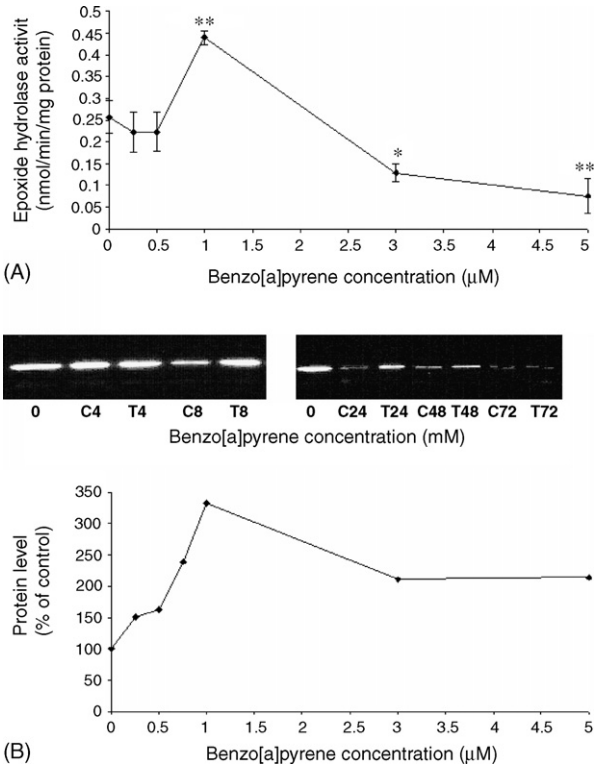

Fig. 8. Concentration-dependent induction of microsomal epoxide hydrolase by benzo[a]pyrene in rat precision-cut lung slices. Precision-cut lung slices were incubated in the presence of a range of benzo[a]pyrene concentrations (0–5 μM) for 24 h. At the end of the incubation period, slices were removed and microsomes isolated: (A) epoxide hydrolase activity assessed using benzo[a]pyrene 4,5-oxide as substrate; (B) apoprotein levels in pooled slices probed with antibodies to epoxide hydrolase. Into each well 234 μg (control) or 117 μg (benzo[a]pyrene-treated) of protein was loaded. Where appropriate, results are expressed as mean ± S.D. of triplicate pools of slices. Plot of apoprotein levels has been corrected for protein. $^{*}P < 0.05$.

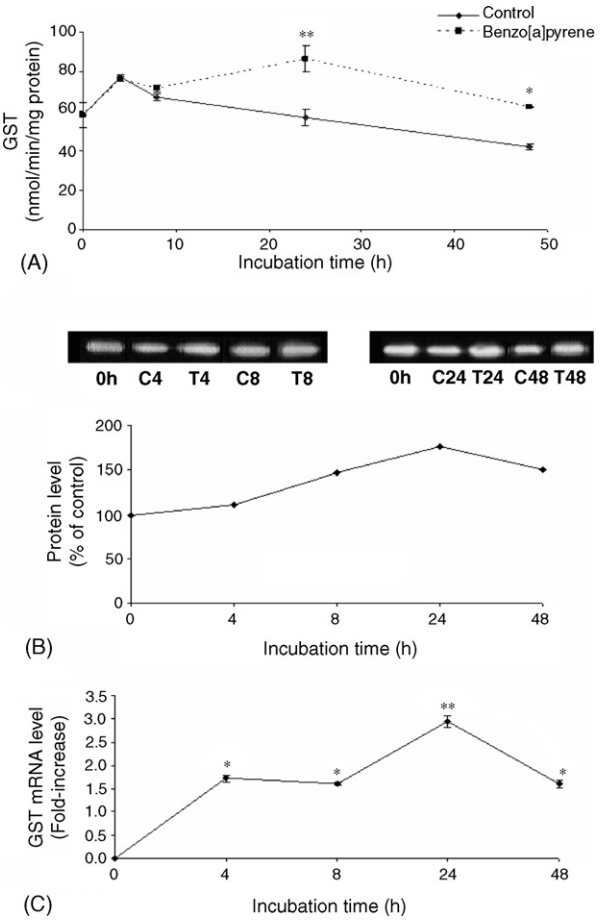

Fig. 9. Time-dependent induction of cytosolic glutathione *S*-transferase by benzo[a]pyrene in rat precision-cut liver slices. Precision-cut liver slices were incubated in the presence and absence of benzo[a]pyrene (10 μM) for various periods of time up to 48 h. At the end of each time period, slices were removed and mRNA or cytosolic fraction isolated: (A) Glutathione *S*-transferase activity assessed using 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole as substrate; (B) apoprotein levels in pooled slices probed with antibodies to glutathione *S*-transferase (Ya) with all wells being loaded with 5 μg of cytosolic protein, where C refers to control and T to benzo[a]pyrene-treated slices, and number denotes period of incubation in hours; (C) fold-increase in mRNA levels compared to controls where slices were incubated with the corresponding volume of DMSO, the vehicle. Where appropriate, results are expressed as mean ± S.D. of triplicate pools of slices. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

been achieved when the slices were incubated with benzo[a]pyrene for 24 h (Fig. 9B); at the mRNA level, elevated levels were observed as early as 4 h, but maximum increase was observed following a 24-h incubation (Fig. 9C). When liver slices were exposed to a range of benzo[a]pyrene concentrations, a statistically significant increase in activity was achieved at the 5–10 μM concentration (Fig. 10A); similarly, apoprotein levels increased maximally at the 10 μM concentration (Fig. 10B). A significant increase in mRNA levels was noted at all concentrations employed, but maximum elevation was attained at a concentration of 50 μM (Fig. 10C). In lung slices, no activity was detectable when DCNB or 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole were used as substrates (results not shown). Activity was present when CDNB served as the accepting substrate, but it was not inducible by exposure to benzo[a]pyrene (1 μM) (Fig. 11). Increasing the concentration to 10 μM had no impact on the conjugation of CDNB with glutathione (results not shown).

4. Discussion

Basal activities of all enzymes studied declined with incubation time in both lung and liver slices as

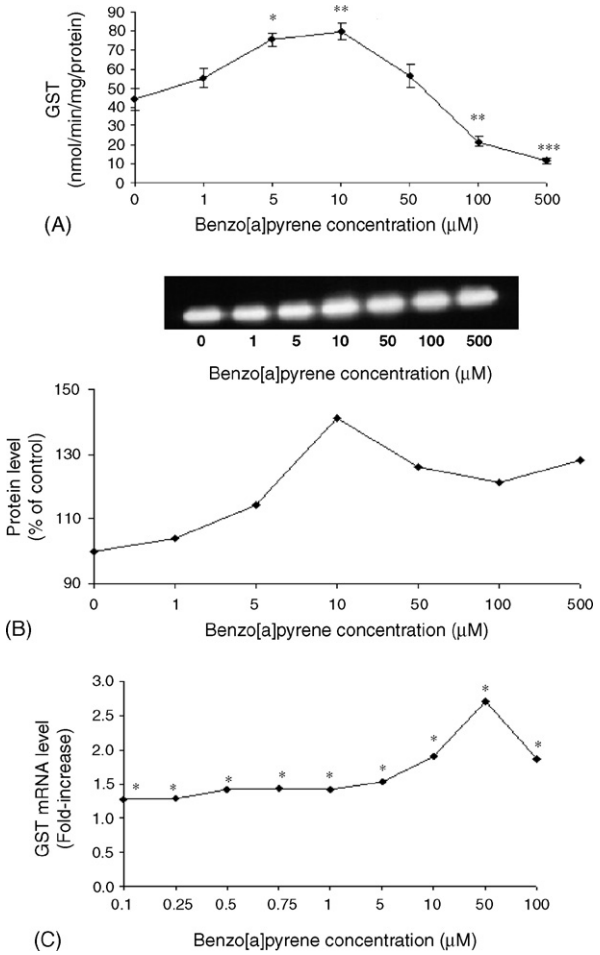

Fig. 10. Concentration-dependent induction of cytosolic glutathione *S*-transferase by benzo[a]pyrene in rat precision-cut liver slices. Precision-cut liver slices were incubated in the presence of a series of concentrations of benzo[a]pyrene (0–500 μM) for 24 h. At the end of this time period, slices were removed and mRNA or cytosolic fraction isolated: (A) Glutathione *S*-transferase activity assessed using 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole as substrate; (B) apoprotein levels in pooled slices probed with antibodies to glutathione *S*-transferase (Ya) with 5 μg of cytosolic protein being loaded in each well; (C) fold-increase in mRNA levels compared to controls where slices were incubated with the corresponding volume of DMSO, the vehicle. Where appropriate, results are expressed as mean ± S.D. of triplicate pools of slices. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

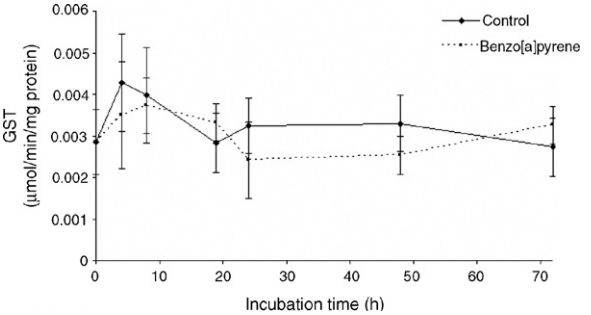

Fig. 11. Time-dependent modulation of cytosolic glutathione *S*-transferase by benzo[a]pyrene in rat precision-cut lung slices. Precision-cut lung slices were incubated in the presence and absence of benzo[a]pyrene (1 μM) for various periods of time up to 72 h. At the end of each time period, slices were removed and cytosolic fraction isolated. Glutathione *S*-transferase activity was assessed using CDNB as substrate. Results are expressed as mean ± S.D. of triplicate pools of slices.

we have previously reported (Hashemi et al., 1999b; Umachandran and Ioannides, 2006).

Ethoxyresorufin *O*-deethylase activity is widely employed as a selective marker for CYP1A1, although to a much lesser extent its metabolism is also catalysed by the other members of the CYP1 family (Namkung et al., 1988). As we have previously reported (Hashemi et al., 2000), ethoxyresorufin *O*-deethylase activity in rat slices declined on incubation, with very little activity being detectable after 48 h, and this was accompanied by a similar loss in CYP1A apoprotein levels. Exposure of the slices, however, to benzo[a]pyrene led to a marked, increase in activity, the highest rise occurring after a 24-h incubation, similar to observations made *in vivo* following treatment of rats with this compound (Iwasaki et al., 1986). A rise in this activity in rat slices has already been reported following incubation with other CYP1A1 inducers such as β-naphthoflavone (Müller et al., 1996), TCDD (Drahushuk et al., 1996) and Aroclor 1254 (Price et al., 2004). In one study, where different incubation time points were employed, all longer than 24 h, level of induction by TCDD was found to increase with time of incubation being highest after 96 h of incubation (Drahushuk et al., 1999), in contrast to the present study where maximum induction was noted after a 24-h exposure to benzo[a]pyrene. In the study of Drahushuk et al. (1999), however, the dynamic organ culture system was employed, whereas in the present study the multiwell plate system was used and this may have influenced the uptake of the inducing agent. Alternatively, induction profile may be dependent on the nature of the inducing agent. In the present studies, induction of ethoxyresorufin *O*-deethylase was concentration dependent reaching a maximum and then declining, in agreement with previous studies (Price et al., 2004). As the rise in activity is accompanied by a rise in apoprotein and mRNA levels, it may be inferred that increased CYP1A1 activity is a consequence of increased enzyme availability resulting from elevated mRNA levels, the result of enhanced transcription and/or mRNA stabilisation. The decline in the extent of ethoxyresorufin *O*-deethylase induction noted at the

higher concentrations of benzo[a]pyrene was also paralleled by a similar decline in apoprotein and mRNA levels, implying that the lower inductive effect represents impaired protein synthesis resulting from the toxicity of the hydrocarbon, although this interpretation requires experimental confirmation. A likely scenario is that at the high benzo[a]pyrene concentrations, the generation of the reactive intermediates, such as dihydrodiol epoxides and quinones (Pelkonen and Nebert, 1982), overwhelms the detoxication pathways leading to toxicity and impaired slice viability.

No suitable substrate probe is currently available for monitoring CYP1B1 activity; studies using immunoblotting could not consistently detect CYP1B1 expression in rat liver slices, especially after prolonged incubation, indicating that this enzyme is unlikely to be a major catalyst of xenobiotic metabolism in this tissue (results not shown). Consistent with such a hypothesis, previous studies have demonstrated that CYP1B1 is poorly expressed in the liver but predominates in the adrenals (Walker et al., 1995). Consequently, the effect of benzo[a]pyrene was evaluated only at the mRNA level. A rise in CYP1B1 mRNA levels was noted as early as after 4 h of incubation with benzo[a]pyrene, with the effect being more pronounced after longer incubations. This is again consistent with previous studies employing rat hepatocytes where CYP1B1 mRNA levels were elevated following exposure to polycyclic aromatic hydrocarbons (Piscaglia et al., 1999). Clearly, although CYP1B1 is poorly expressed in the liver, it is inducible, at least at the mRNA level, in response to xenobiotic exposure.

Modulation of CYP1A1/1B1 by benzo[a]pyrene was also investigated in precision-cut rat lung slices at the activity and/or apoprotein level. CYP1A1 activity, monitored using ethoxyresorufin *O*-deethylase, declined following prolonged culture periods; treatment with benzo[a]pyrene led to a marked increase in activity, especially after a 48-h incubation, confirming previous observations (Price et al., 2004). In the present studies using the multiwell system, induction was maximal at 1 μM concentration, but marked induction by benzo[a]pyrene was demonstrated at higher concentrations when the dynamic organ culture system was used (Price et al., 2004). However, when β-naphthoflavone was used as the CYP1A1 inducer, the level of induction diminished at the higher concentrations (Lake et al., 2003). As the CYP1A1 apoprotein levels were also elevated following incubation of the slices with benzo[a]pyrene, it may be inferred that, similar to the liver, increased CYP1 activity is the consequence of increased enzyme availability. CYP1B1 expression in the lung is higher than the liver, so expression could be monitored by Western blotting. CYP1B1 expression was similarly up-regulated in lung slices treated with benzo[a]pyrene.

One of the objectives of this study was to investigate whether epoxide hydrolase and glutathione *S*-transferase are inducible in precision-cut liver and lung slices, as has been shown for cytochromes P450. Clearly, both of these enzymes were inducible in rat hepatic slices, following incubation for 24 h with benzo[a]pyrene, and the increase in expression was evident at the activity, apoprotein and mRNA level. Transcriptional activation appears to occur quite rapidly after exposure of the slices to benzo[a]pyrene, but several hours are required for mRNA to attain maximum levels and be translated into elevated apoprotein levels and a rise in enzyme activity. Induction of epoxide hydrolase and selectively of the Ya subunit of glutathione *S*-transferase in hepatic microsomes following treatment of rats with polycyclic aromatic hydrocarbons has already been documented (Oesch, 1976; Igarashi et al., 1987). Induction of epoxide hydrolase in liver slices appears to be concentration-dependent, with a maximum being achieved at 10 μM concentration; at higher concentrations activity declined, similar to cytochrome P450 expression, presumably as a consequence of toxicity induced by reactive metabolites of the hydrocarbon (Pelkonen and Nebert, 1982). As increases in the activity of these enzymes is accompanied by elevated mRNA and apoprotein levels, it may be inferred, once again, that enhanced enzyme activity is a consequence of increased enzyme synthesis, rather than activation of pre-existing enzyme, and increased transcription appears to be a contributory mechanism, although mRNA stabilization cannot be excluded. Indeed, this is exemplified by the observation that transcript levels of epoxide hydrolase continue to increase even at benzo[a]pyrene concentrations in excess of 10 μM, when enzyme activity and apoprotein levels decline due to presumed toxicity. Moreover, we demonstrated that the induction of glutathione *S*-transferase by benzo[a]pyrene in rat liver slices is isoenzyme-specific since a rise in activity was observed only when 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole was used, a substrate that is employed to monitor Ya activity of the α-family (Ricci et al., 1994) that is regulated by the Ah receptor (Rushmore and Pickett, 1990), for which planar compounds, such as benzo[a]pyrene, serve as strong ligands.

Epoxide hydrolase activity was lower in lung slices compared with liver, in agreement with previous reports (Arand and Oesch, 2002), but was also inducible by benzo[a]pyrene. In liver slices maximal induction required a benzo[a]pyrene concentration of 10 μM,

whereas in lung slices it could be achieved at a far lower concentration of 1 μM. In contrast to the liver, however, no glutathione *S*-transferase activity was detectable in the lungs when 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole was utilised as substrate, presumably as the conjugation of this substrate is selectively catalysed by the alpha class of transferases (Ricci et al., 1994) which is not expressed in the lung (Sherratt and Hayes, 2002). Glutathione *S*-transferase activity in lung slices was detectable only when CDNB was used as the substrate, but was not inducible by benzo[a]pyrene. CDNB is a non-specific substrate, the conjugation of which with glutathione is catalysed by a number of glutathione *S*-transferase isoenzymes, including the mu and pi classes (Ricci et al., 1994), which are expressed in the lung (Sherratt and Hayes, 2002). Although activity using DCNB as substrate in the lung has been detected, this is only about 15% of the hepatic activity, and this may explain the lack of activity in the lung slices (Eke et al., 1996). It is also pertinent to point out that the rate of conjugation of CDNB in rats is far higher, about 20-fold, when compared with DCNB (Eke et al., 1996). Failure to induce this enzyme system in lung slices cannot be attributed to lack of uptake of benzo[a]pyrene since other enzyme systems were clearly up-regulated under identical conditions.

Maximum expression of the various enzymes in lung slices was achieved at a concentration of 1 μM, whereas in the liver it necessitated 10 μM. It is conceivable that, at least in part, this difference in concentration may reflect the higher rate of metabolic breakdown of this compound in the liver compared with lung, leading to a rapid decline in its effective concentration; the rate of metabolism of benzo[a]pyrene to phenolic products in liver slices is higher compared with lung slices (unpublished observations).

In summary, in the present studies we confirm induction of CYP1A1 in rat and lung slices, and demonstrate for the first time that precision-cut tissue slices may be used to evaluate induction of CYP1B1, epoxide hydrolase and glutathione *S*-transferase activities following exposure to chemicals. Use of slices, in preference to primary cell cultures, is particularly useful for studies in lung as this tissue is characterised by a heterogeneous cellular population. When cultured in vitro, tissue slices retain an intact protein synthesis apparatus and can thus respond to enzyme inducers. Using the slice system, it would be relatively facile to screen dietary phytochemicals for their ability to stimulate glutathione *S*-transferase activity in the liver and other tissues and, thus, identify potential chemopreventive agents (Talalay et al., 1995). Moreover, this approach can potentially be extended to human tissues, incubated under the same conditions. Such studies will not only indicate whether this enzyme is inducible in human tissues by a particular phytochemical but will also define the concentrations at which this will be effective, and can thus be related to plasma levels achievable through dietary intake.

Acknowledgements

The authors acknowledge with thanks funding of this work by the European Union through the AMBIPAH project, and thank Dr. M. Arand (University of Zurich, Zurich, Switzerland) for the kind gift of antibodies to epoxide hydrolase.

References

Arand, M., Oesch, F., 2002. Mammalian xenobiotic epoxide hydrolases. In: Ioannides, C. (Ed.), Enzyme Systems that Metabolise Drugs and other Xenobiotics. John Wiley & Sons, Ltd., Chichester, pp. 459–483.

Baumann, P., Kerdar, R.S., Cramer, P., Feser, W., Blode, H., Salomon, A., Kuhnz, W., 1996. Use of rat and human liver slices for the detection of steroid hormone-induced DNA-adducts in vitro by means of the ^{32}P-postlabelling technique. Pharmacol. Toxicol. 78, 214–223.

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Burke, M.D., Mayer, R.T., 1974. Ethoxyresorufin: direct fluorimetric assay of a microsomal *O*-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Met. Disp. 2, 583–588.

Dansette, P.M., DuBois, G.C., Jerina, D.M., 1979. Continuous fluorometric assay of epoxide hydratase activity. Anal. Biochem. 97, 340–345.

De Kanter, R., Olinga, P., De Jager, M.H., Merema, M.T., Meijer, D.K.F., Groothius, G.M.M., 1999. Organ slices as an in vitro test system for drug metabolism in human liver, lung and kidney. Toxicol. In Vitro 13, 737–744.

Drahushuk, A.T., McGarrigle, B.P., Tai, H.L., Kitareewan, S., Goldstein, J.A., Olson, J.R., 1996. Validation of precision-cut liver slices in dynamic organ culture as in vitro model for studying CYP1A1 and CYP1A2 induction. Toxicol. Appl. Pharmacol. 140, 393–403.

Drahushuk, A.T., McGarrigle, B.P., Slezak, B.P., Stegeman, J.J., Olson, J.R., 1999. Time- and concentration-dependent induction of CYP1A1 and CYP1A2 in precision-cut liver slices incubated in dynamic organ culture in the presence of 2,3,7,8-tetrachlordibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 155, 127–138.

Edwards, R.J., Price, R.J., Watts, P.S., Renwick, A.B., Tredger, J.M., Boobis, A.R., Lake, B.G., 2003. Induction of cytochrome P450 enzymes in cultured precision-cut human liver slices. Drug Met. Disp. 31, 282–288.

Eke, C.E., Vural, N., Iscab, M., 1996. Combined effects of ethanol and cigarette smoke on hepatic and pulmonary xenobiotic metabolizing enzymes in rats. Chem. Biol. Interact. 102, 155–167.

Ekins, S., 1996. Past, present, and future applications of precision-cut liver slices for in vitro xenobiotic metabolism. Drug Met. Rev. 28, 591–623.

Guengerich, F.P., 2003. Cytochrome P450 oxidations in the generation of reactive electrophiles: epoxidation and related reactions. Arch. Biochem. Biophys. 409, 59–71.

Guengerich, F.P., Johnson, W.W., Shimada, T., Ueng, Y.-F., Yamazaki, H., Langouët, S., 1998. Activation and detoxication of aflatoxin B_1. Mut. Res. 402, 121–128.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione *S*-transferase, the first enzymic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.

Harrigan, J.A., McGarrigle, B.P., Sutter, T.R., Olso, J.R., 2006. Tissue specific induction of cytochrome P450 (CYP) 1A1 and 1B1 in rat liver and ling following in vitro (tissue slice) and in vivo exposure to benzo[a]pyrene. Toxicol. In vitro 20, 426–438.

Hashemi, E., Dobrota, M., Till, C., Ioannides, C., 1999a. Structural and functional integrity of precision-cut liver slices in xenobiotic metabolism: a comparison of the dynamic organ and multiwell plate culture procedures. Xenobiotica 29, 11–25.

Hashemi, E., Till, C., Ioannides, C., 1999b. Stability of phase II conjugation systems in cultured precision-cut rat hepatic slices. Toxicol. In Vitro 13, 459–466.

Hashemi, E., Till, C., Ioannides, C., 2000. Stability of cytochrome P450 in cultured precision-cut rat liver slices. Toxicology 149, 51–61.

Herrero, M.E., Arand, M., Hengstler, J.G., Oesch, F., 1997. Recombinant expression of human microsomal epoxide hydrolase protects V79 Chinese hamster cells from styrene oxide- But not from ethylene oxide-induced DNA strand breaks. Environ. Mol. Mutagen. 30, 429–439.

Igarashi, T., Irokawa, N., Ono, S., Ohmori, S., Ueno, K., Kitagawa, H., Satoh, T., 1987. Difference in the effects of phenobarbital and 3-methylcholanthrene treatment on subunit composition of hepatic glutathione-*S*-transferase in male and female rats. Xenobiotica 17, 127–137.

Ioannides, C., Parke, D.V., 1990. The cytochrome P-450 I gene family of microsomal haemoproteins and their role in the metabolic activation of chemicals. Drug Met. Rev. 22, 1–85.

Iwasaki, K., Lum, P.Y., Ioannides, C., Parke, D.V., 1986. Induction of cytochrome P-448 activity as exemplified by the *O*-deethylation of ethoxyresorufin. Effects of dose, sex, tissue and animal species. Biochem. Pharmacol. 35, 3879–3884.

Lake, B.G., Beamand, J.A., Japenga, A.C., Renwick, A., Davies, S., Price, R.J., 1993. Induction of cytochome P-450-dependent enzyme activities in cultured rat slices. Food Chem. Toxicol. 31, 377–386.

Lake, B.G., Charzat, C., Tredger, J.M., Renwick, A.B., Beamand, J.A., Price, R.J., 1996. Induction of cytochrome P-450 isoenzymes in cultured rat and human precision-cut liver slices. Xenobiotica 26, 297–306.

Lake, B.G., Beamand, J.A., Tredger, J.M., Barton, P.T., Renwick, A.B., Price, R.J., 1999. Inhibition of xenobiotic-induced genotoxicity in cultured precision-cut rat liver slices. Mut. Res. 440, 91–100.

Lake, B.G., Meredith, C., Scott, M.P., Renwick, A.B., Price, R.J., 2003. Use of cultured precision-cut rat lung slices to study the in vitro induction of pulmonary cytochrome P450 forms. Xenobiotica 33, 691–702.

Lerche-Langrand, C., Toutain, H.J., 2000. Precision-cut liver slices: characteristics and use for in vitro pharmaco-toxicology. Toxicology 153, 221–253.

Martignoni, M., de Kanter, R., Grossi, P., Mahnke, A., Saturno, M., Monshower, M., 2004. An in vivo and in vitro comparison of CYP induction in rat liver and intestine using slices and quantitative RT-PCR. Chem. Biol. Interact. 151, 1–11.

Meredith, C., Scott, M.P., Renwick, A.B., Price, R.J., Lake, B.G., 2003. Studies on the induction of rat hepatic CYP1A, CYP2B, CYP3A and CYP4A subfamily form mRNAs in vivo and in vitro using precision-cut rat liver slices. Xenobiotica 33, 511–527.

Miller, M.G., Beyer, J., Hall, G.L., De Graffenried, A., Adams, P.E., 1993. Predictive value of liver slices for metabolism and toxicity in vivo: use of acetaminophen as a model hepatotoxicant. Toxicol. Appl. Pharmacol. 122, 108–116.

Moronvalle-Halley, V., Sacre-Salem, B., Sallez, V., Labbe, G., Gautier, J.C., 2005. Evaluation of cultured, precision-cut rat liver slices as a model to study drug-induced liver apoptosis. Toxicology 207, 203–214.

Müller, D., Glöckner, R., Rost, M., 1996. Monooxygenation, cytochrome P4501A1 and P4501A1-mRNA in rat liver slices exposed to beta-naphthoflavone and dexamethasone in vitro. Exp. Toxic. Pathol. 48, 433–438.

Müller, D., Steinmetzer, P., Pissowotzki, K., Glöckner, R., 2000. Induction of cytochome P450 2B1-mRNA and pentoxyresorufin *O*-dependentylation after exposure of precision-cut rat liver slices to Phenobarbital. Toxicology 144, 93–97.

Namkung, M.J., Yang, H.L., Hulla, J.E., Juchau, M.R., 1988. On the substrate specificity of cytochrome P450IIIA1. Mol. Pharmacol. 34, 628–637.

Oesch, F., 1976. Differential control of rat microsomal arylhydrocarbon monooxygenase and epoxide hydratase. J. Biol. Chem. 251, 79–87.

Onderwater, R.C.A., Commandeur, J.N.M., Roseboom, M., Vermeulen, N.P.E., 2005. Uptake-toxicity relationships of a series of N-substituted N′-(4-imidazole-ethyl) thiourea in precision-cut rat liver slices. Xenobiotica 35, 391–404.

Pelkonen, O., Nebert, D.W., 1982. Metabolism of polycyclic aromatic hydrocarbons: etiologic role in carcinogenesis. Pharmacol. Rev. 34, 189–222.

Pfaff, M., Pogawa, N., Akinci, S., Schutz, W., Wiegand, S., Kummer, W., Wess, J., HaberbergerF R.V., 2005. Activation of the SPHK/SIP signalling pathway is coupled to muscarinic receptor-dependent regulation of peripheral airways. Respir. Res. 6 (Article No 48).

Piscaglia, F., Knittel, T., Kobold, D., Barnikol-Watanabe, S., Di Rocco, P., Ramadori, G., 1999. Cellular localization of hepatic cytochrome 1B1 expression and its regulation by aromatic hydrocarbons and inflammatory cytokines. Biochem. Pharmacol. 58, 157–165.

Price, R.J., Mistry, H., Wield, P.T., Renwick, A.B., Beamand, J.A., Lake, B.G., 1996. Comparison of the toxicity of allyl alcohol, coumarin and menadione in precision-cut rat, guinea-pig, cynomolgus monkey and human liver slices. Arch. Toxicol. 71, 107–111.

Price, R.J., Renwick, A.B., Walters, D.G., Young, P.J., Lake, B.G., 2004. Metabolism of nicotine and induction of CYP1A forms in precision-cut rat liver and lung slices. Toxic. In Vitro 18, 179–185.

Ricci, G., Caccuri, A., Bello, M., Pastore, A., Piemonte, F., Federici, G., 1994. Colorimetric and fluorometric assays of glutathione-*S*-transferase based on 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole. Anal. Biochem. 218, 463–465.

Rushmore, T.H., Pickett, C.B., 1990. Transcriptional regulation of the rat glutathione *S*-transferase-Ya subunit gene-characterization of a xenobiotic-responsive element controlling inducible expression by phenolic antioxidants. J. Biol. Chem. 265, 14648–14653.

Sherratt, P.J., Hayes, J.D., 2002. Glutathione *S*-transferases. In: Ioannides, C. (Ed.), Enzyme Systems that Metabolise Drugs and other Xenobiotics. John Wiley & Sons, Ltd., Chichester, pp. 319–352.

Talalay, P., Fahey, J.W., Holtzclaw, W.D., Prestera, T., Zhang, Y.S., 1995. Chemoprotection against cancer by Phase 2 enzyme induction. Toxicol. Lett. 82–83, 173–179.

Umachandran, M., Howarth, J., Ioannides, C., 2004. Metabolic and structural viability of precision-cut rat lung slices in culture. Xenobiotica 34, 771–780.

Umachandran, M., Ioannides, C., 2006. Stability of cytochromes P450 and phase II conjugation systems in precision-cut lung slices cultured up to 72 h. Toxicology 224, 14–21.

van de Bovenkamp, M., Groothuis, G.M.M., Draaisma, A.L., Merema, M.T., Bezuijen, J.I., van Gils, M.J., Meijer, D.K.F., Friedman, S.L., Olinga, P., 2005. Precision-cut liver slices as a model to study toxicity-induced hepatic stellate cell activation in a physiological milieu. Toxicol. Sci. 85, 632–638.

Walker, N.J., Gastel, H.A., Costa, L.T., Clark, G.C., Lucier, G.W., Sutter, T.R., 1995. Rat CYP1B1: an adrenal cytochrome P450 that exhibits sex-dependent expression in livers and kidneys of TCDD-treated animals. Carcinogenesis 16, 1319–1327.