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Up-regulation of CYP1A/B in rat lung and liver, and human liver precision-cut slices by a series of polycyclic aromatic hydrocarbons; association with the Ah locus and importance of molecular size

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Abstract

Exposure of precision-cut rat liver slices to six structurally diverse polycyclic aromatic hydrocarbons, namely benzo[*a*]pyrene, benzo[*b*]fluoranthene, dibenzo[*a*,*h*]anthracene, dibenzo[*a*,*l*]pyrene, fluoranthene and 1-methylphenanthrene, led to induction of ethoxyresorufin *O*-deethylase, CYP1A apoprotein and CYP1A1 mRNA levels, but to a markedly different extent. In liver slices, constitutive CYP1A1 mRNA levels were higher, as well as being markedly more inducible by PAHs, compared with CYP1B1, a similar profile to that observed in human liver slices following exposure to the PAHs. Increase in ethoxyresorufin *O*-deethylase and in CYP1A1 apoprotein levels was also observed when precision-cut rat lung slices were incubated with the same PAHs, the order of induction potency being similar to that observed in liver slices. Under the same conditions of exposure, CYP1B1 apoprotein levels were elevated in the lung. Up-regulation of CYP1A1 by the six PAHs correlated with their affinity for the Ah receptor, determined using the chemical-activated luciferase expression (CALUX) assay. It may be concluded that (a) precision-cut liver and lung slices may be used to assess the CYP1 induction potential of chemicals at the activity, apoprotein and mRNA levels; (b) rat is a promising surrogate animal for human in studies to evaluate CYP1 induction potential; (c) CYP1A1 is far more inducible than CYP1B1 in both rat liver and lung; (d) CYP1 up-regulation by PAHs is related to their affinity for the Ah receptor, and finally (e) computer analysis revealed that the ratio of molecular length/width is an important determinant of CYP1 induction potency among equiplanar PAHs.

Keywords: Precision-cut slices; Polycyclic aromatic hydrocarbons; CYP1A; CYP1B; Cytochromes P450; Enzyme induction

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) comprise one of the largest and most ubiquitous classes of environmental chemical carcinogens. The major sources of human exposure are diet, as these are formed during domestic cooking, air and tobacco smoking (Skog and Jägerstad, 1998). They are indirect-acting genotoxic carcinogens in that they manifest their carcinogenicity through reactive intermediates that are produced following metabolic activation catalysed in many tissues, but most prominently in the liver.

The principal pathway of metabolic activation of PAHs proceeds through an initial cytochrome P450-mediated bioactivation to generate epoxides which are converted to the corresponding *trans*-dihydrodiols by epoxide hydrolase; finally, a second oxidation, also catalysed by cytochromes P450, yields the dihydrodiol-epoxide, the ultimate carcinogen (Conney, 1982). The cytochrome P450 family responsible for

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the metabolism, including bioactivation, of PAHs is CYP1, in particular CYP1A1 and CYP1B1 (Shimada and Fujii-Kuriyama, 2004; Ioannides and Lewis, 2004). Both of these enzymes are constitutively poorly expressed in the liver, and are essentially extrahepatic enzymes (Guengerich, 1990; Bhattacharyya et al., 1995). CYP1, however, is probably the most inducible CYP family, at least in terms of induction observed, being up-regulated by planar compounds in the liver and extrahepatic tissues of animals and humans (Ioannides and Parke, 1990; Christou et al., 1995). This up-regulation of CYP1 is regulated by the Ah (aryl hydrocarbon) receptor, and a number of studies have shown that CYP1-inducing PAHs are good ligands for this receptor (Cheung et al., 1993; Machala et al., 2001; Piskorska-Pliszczynska et al., 1986).

The current studies were conducted in precision-cut slices in order to facilitate the use of human tissue. The advantages of adopting this *in vitro* system, in comparison with other systems such as subcellular fractions and primary hepatocytes, have frequently been pointed out in reviews (De Kanter et al., 1999; Lerche-Langrand and Toutain, 2000). Slices have been prepared from a number of tissues including liver (Hashemi et al., 1999), lung (Umachandran et al., 2004), intestine and colon (Van de Kerkhof et al., 2005).

The objectives of the present study were to (a) assess the ability of six structurally diverse PAHs to up-regulate the two CYP1 enzymes involved in their metabolism, namely CYP1A1/B1, in the liver and lung, the principal site of their bioactivation and a target tissue, respectively; (b) establish whether precision-cut liver slices can be used to assess the potential of PAHs to modulate CYP1A1/B1; (c) evaluate whether rat is an appropriate surrogate animal for human in such studies by investigating the ability of the same series of PAHs to modulate CYP1A1 in human liver slices; (d) investigate whether up-regulation of the same enzyme systems involves transcriptional activation mediated through the Ah receptor; and finally (e) through computer analysis identify structural characteristics that are associated with the induction of the CYP1 enzymes.

2. Materials and methods

2.1. Materials

Dibenzo[*a*,*l*]pyrene, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 1-methylphenanthrene (LGC Promochem, Middlesex, UK), rat genomic DNA (Novagen, WI, USA), RNase-free DNase, cell culture lysis reagent, luciferase assay reagent (Promega, WI, USA), NADPH, sulphatase, β-glucuronidase, β-naphthoflavone, benzo[*a*]pyrene, fluoranthene, benzo[*b*]fluoranthene, dibenzo[*a*,*h*]anthracene, ethoxyresorufin, resorufin, 7-ethoxycoumarin, 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, hexamers, Superscript II and RPMI 1640 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, recognising both CYP1A1 and CYP1A2, and anti-CYP1B1 antibodies were obtained from BD Biochemicals (Oxford, UK).

2.2. Preparation and incubation of precision-cut tissue slices

Liver sections from two human cadaveric livers that could not be used for transplantation purposes were obtained from the UK Human Tissue Bank (The Innovation Centre, Oxford Street, Leicester, UK). Donor 1 was a 60-year-old male and Donor 2 a 57-year-old female; both were Caucasian and smokers. Sections were received 8–12 h after the liver was removed from the donor and were transported in cold University of Wisconsin (UW) preservation solution on ice. On receipt, the liver sections were immediately transferred into a sterile container and, after the UW solution was carefully decanted, they were washed 3–4 times with culture medium. Slices were prepared as with rat liver (*vide infra*) and pre-incubated for 30 min prior to the start of the experiment, to allow equilibrium to be reached and ensure complete removal of the transport buffer. Metabolic viability was evaluated using 7-ethoxycoumarin as substrate, following a 6-h incubation (*vide infra*). 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:dark cycle with light onset at 07.00 h. Rats were killed by cervical dislocation, and liver was immediately excised. In the *in vivo* study, rats were treated with a single intraperitoneal dose of either β-naphthoflavone or benzo[*a*]pyrene (25 mg/kg) and were killed 24 h afterwards.

Rat and human 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., 1999). The multiwell plate procedure, using 12-well culture plates, was used to culture the slices. 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; Umachandran and Ioannides, 2006). A pre-incubation of 60 min was carried out. For incubations