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Short Communication: The effect of CYP1A1 induction on the formation of benzo[*a*]pyrene adducts in liver and lung DNA and plasma albumin in rats exposed to benzo[*a*]pyrene: adduct quantitation by immunoassay and an HPLC method

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Induction of cytochrome P450 enzymes by exposure to polycyclic aromatic hydrocarbons (PAH) can result in both decreased or increased PAH adduct levels. The lung is a main target site for PAH-carcinogenesis. By HPLC determination of B[*a*]P-*r*-7, *t*-8-dihydrodiol, *t*-9,10-epoxide (BPDE-I)–DNA adducts in rat, the level of the ultimate carcinogenic B[*a*]P-metabolite was higher in lungs than in liver. However, measured by immunoassay, the total benzo[*a*]pyrene (B[*a*]P)–DNA adduct levels were higher in liver than in lungs. Induction of CYP1A1 *in vivo* in rat by repeated i.p. doses of methylcholanthrene (MC) prior to a single dose of B[*a*]P resulted in a 2·4 times increase in CYP1A1 activity in liver tissue and 1·5 times higher levels of total B[*a*]P–DNA adducts in lung and liver compared with controls which only received B[*a*]P. Increased levels of BPDE-I–DNA adducts were significantly correlated to increased CYP1A1 activity in induced lung tissue but not in liver. The times to reach maximum adduct levels were similar for both controls and MC-induced rats in both lung and liver, and plasma albumin. The BPDE-I–albumin adducts reached a maximum level around 1 day after B[*a*]P exposure and could not be used as a reliable marker of the short term PAH exposure in this study.

Keywords: DNA adducts, protein adducts, polycyclic aromatic hydrocarbons, ELISA, HPLC.

Abbreviations: PAH, polycyclic aromatic hydrocarbons; BPDE, benzo[*a*]pyrene-7,8-diol-9,10-oxide; BPDE-I, benzo[*a*]pyrene-*r*-7, *t*-8-dihydrodiol, *t*-9,10-epoxide; MC, methylcholanthrene; i.p., intraperitoneal; P450, microsomal cytochrome P450s; CYP, cytochrome P450 protein; EROD, 7-ethoxyresorufin *O*-deethylation activity; ELISA, enzyme linked immunoassay.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are formed by incomplete combustion of organic matter and fossil fuels, and are widely distributed throughout the environment. Several PAH compounds are known or suspected carcinogens in both animals and humans (IARC 1983a, b). Environmental and occupational exposure to mixtures of PAHs results in varying tumorigenic responses compared with the effect of each compound individually (Weyand *et al.* 1995, Arif and Gupta 1996).

Several PAHs are metabolically activated to reactive diol-epoxides by cytochrome P450 (CYP) enzymes and epoxide hydrolase. The most extensively

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studied PAH, benzo[*a*]pyrene (B[*a*]P), has been used as an indicator for carcinogenic PAHs in complex mixtures (Gammage 1983, IARC 1983). B[*a*]P-7,8-diol is metabolized by CYP1A1 to the ultimate carcinogenic form B[*a*]P-*r*-7, *t*-8-dihydrodiol, *t*-9,10-epoxide (BPDE-I), which binds covalently to DNA and proteins resulting in B[*a*]P adducts (Dipple *et al.* 1984, Day *et al.* 1994). The formation of DNA-adducts appears to be the essential initiation step in PAH-induced cancers (Gelboin 1980, Harvey 1980, Stowers and Anderson 1985).

PAHs induce tumours in various tissues of rodents, regardless of route of administration (Stowers and Anderson 1985). The lung is one of the main target sites of PAH-induced carcinogenesis, although the total and specific activities of cytochrome P450 are much higher in the liver, as reported in Wall *et al.* (1991). The mechanisms of tissue specific cancer induction are still not well known, but include uptake, formation of various metabolite–DNA adducts and the persistence of adducts in tissue with slow cell turnover rates (Stowers and Anderson 1985, Hall and Grover 1990). To further investigate these mechanisms, we have studied organ differences in PAH-adduct levels in relation to the effects of cytochrome P450 induction.

Frequently used methods for determination of B[*a*]P–DNA adducts are ^{32}P-postlabelling or immunoassay (Rojas *et al.* 1994, Hemminki 1995). Recently a high-pressure liquid chromatography (HPLC)/fluorometric method was developed by Alexandrov *et al.* (1992) for measuring BPDE–DNA adducts in human tissue by quantitating B[*a*]P-tetrols released after acid hydrolysis of DNA adducts. Furthermore, since DNA from the target organ is usually not readily accessible in human biomonitoring, it is valuable to study carcinogen–DNA and –protein adduct levels in blood as surrogate dosimeters for DNA adducts in target tissue (Hemminki 1995, Meyer and Bechtold 1996).

Since several enzymes are involved in the PAH metabolic pathway, enzyme inducibility may be an important risk factor for cancer after PAH exposure. Methylcholanthrene (MC) is a PAH that has been shown to increase the rate of B[*a*]P metabolite formation in hepatic microsome preparations (Yang *et al.* 1997). However, MC pre-treatment decreased the BPDE–DNA adduct level in lungs from B[*a*]P-exposed mice (Bjelogrlic *et al.* 1993). Hughes and Phillips (1990) found evidence for both synergistic and inhibitory effect on DNA-adduct formation of different dibenzpyrenes and B[*a*]P when applied in combination to mouse skin. Although the induction of cytochrome P450 and the formation of PAH adducts have been studied in several *in vitro* studies, *in vivo* studies are rare.

In the present study we have investigated the effect of induction of CYP1A1 by repeated doses of MC on the *in vivo* formation of B[*a*]P–DNA adducts in rat liver and lung tissue and B[*a*]P–protein adducts in plasma albumin by immunoassay. For comparison, BPDE-I bound to DNA and albumin was analysed by an HPLC method. CYP1A1 activity was measured in rat liver microsomes at several time points and correlated to adduct formation.

Materials and methods

Chemicals and reagents

Benzo[*a*]pyrene (CAS No. 50-32-8), 7-ethoxyresorufin, glucose-6-phosphate, 4-methylumbelliferyl phosphate, bisbenzimide (Hoechst 33258), RNase A, calf thymus DNA, β-NADP and lipase (II) were purchased from Sigma Chemical Co. (St Louis, MO). Proteinase K and glucose-6-phosphate dehydrogenase were supplied by Boehringer Mannheim (Mannheim, Germany). Folin-Ciocalteu phenol reagent was supplied by E.Merck (Darmstadt, Germany), and 3-methylcholanthrene (CAS No.

56-49-5) and resorufin by Fluka Chemie AG (Buchs, Switzerland). Alkaline phosphatase-conjugated goat anti-rabbit IgG $F(ab)_2$ was obtained from Jackson Inc. (Avondale, PA). The four isomers of benzo[*a*]pyrene-7,-8, 9,10-tetrahydrotetrols (CAS No. 62697-16-9; 62697-19-2; 62697-13-6; 62697-17-0) were obtained from NCI Chemical Carcinogen Repository (Midwest Research Institute, Kansas City, MO). Microtitre plates (polyvinyl chloride fastbinder 6695) were purchased from Costar Ltd (Badhoevedorp, The Netherlands). Hypnorm® (fluanisonum/fentanyli) was purchased from Janssen Pharmaceutica NV (Beerse, Belgium) and Dormicum (Midazolam) from F. Hoffmann-La Roche AG (Basel, Switzerland). The corn-oil was produced by Matheson, Coleman and Bell®. Low-modified BPDE calf thymus DNA (12·5 fmol μg^{-1}) was prepared essentially after a procedure by Newman *et al.* (1988). High-modified BPDE calf thymus DNA (36 pmol μg^{-1}, 20 $\mu g\ ml^{-1}$ DNA) and polyclonal rabbit antibody (MP33) were generously provided by Dr A. Weston and Dr M. Poirier, respectively.

The following nomenclature for benzo[*a*]pyrene–DNA adducts has been used: total B[*a*]P–DNA adducts for adducts quantitated with immunoassay (ELISA) since several B[*a*]P–DNA adducts may be detected by immunoassay; BPDE-I–DNA adducts for adducts quantitated by the HPLC method since the main BPDE-I–DNA adduct detected and the only adduct quantitated by HPLC was B[*a*]P-*r*-7, *t*-8, *t*-9, *c*-10-tetrahydrotetrol.

Animals and chemical treatments

Fifty-two male Wistar rats (Mol:WIST, weight approximately 250 g) were fed a standard diet (B&K Universal AS, Norway) and water *ad libitum*. A group of 26 rats was given i.p. doses of 25 mg kg^{-1} MC dissolved in 0·6 ml corn-oil for four consecutive days, the other group of 26 rats was given the same i.p doses of corn-oil only (control group). Daily injections of 25 mg kg^{-1} body weight for 4 days were required for maximal induction *in vivo* (Rodrigues and Prough 1991). On the fourth day all the rats were given 100 mg kg^{-1} B[*a*]P (Ross *et al.* 1990) i.p. in corn-oil. To assure correct deposition of the test compounds the rats were given light anaesthesia with 0·1 ml per 100 g b.w. s.c. Hypnorm/Dormicum solution (1:1 in equal volume of water) before each i.p. injection. Rats were sacrificed in groups of six on days 1, 2 and 4, and in groups of four on days 7 and 11, after the B[*a*]P injection. The rats were anaesthetized with 0·2 ml per 100 g s.c. Hypnorm/Dormicum solution and lungs and liver were surgically removed and stored at –20 °C. Approximately 10 ml of blood was drawn from the aorta of each animal with a heparinized syringe. Plasma, buffy coat and red blood cells were separated by centrifugation (1200 *g* for 15 min) and stored at –20 °C.

Microsomal P450 enzyme activity

CYP1A1 enzyme activity was determined in liver microsomes by single endpoint fluorometric measurement of 7-ethoxyresorufin *O*-deethylation activity (EROD). The procedure was essentially as described in Lake (1987). A microsomal supernatant was obtained by homogenizing liver tissue (1·0 g) in 0·15 M KCl and 50 mM Tris–HCl, pH 7·4 and centrifugation at 10 000 *g* for 20 min (4 °C) and by further sentrifugation at 100 000 *g* for 60 min (4 °C). The microsomal pellet was washed and re-suspended in 3 ml 0·15 M KCl and 50 mM Tris–HCl buffer, pH 7·4. Samples of 500 µl were used for protein quantitation (Lowry *et al.* 1951) and the remaining microsomes were stored at –70 °C after addition of glycerol to a final concentration of 20%. Enzyme activity was expressed in nmol min^{-1} mg^{-1} protein.

DNA isolation

DNA from 1·0 g tissue was isolated essentially as described in Beach and Gupta (1992). DNA concentration was quantitated spectrophotometrically and by fluorescence measurements with a Hoechst 33258 instrument (Labarca and Paigen 1980). The isolated DNA was divided in two batches for analysis by immunoassay and an HPLC method.

B[a]P–DNA adduct determination in lung and liver tissue by competitive ELISA

The ELISA method was performed essentially as USERIA (Santella *et al.* 1988, Øvrebø *et al.* 1992) except for the use of 4-methylumbelliferyl phosphate instead of ^{3}H-PNP as alkaline phosphate substrate. For detailed description of the procedure see Øvrebø *et al.* (1992). 4-Methylumbelliferyl phosphate was incubated for 2 h at 37 °C. The reaction was stopped by removing 50 µl from the well and diluting to 1 ml with distilled water. Fluorescence was measured with a spectrofluorophotometer (Shimadzu RF-5000) with an excitation wavelength of 360 nm and emission at 450 nm. Low-modified BPDE–DNA was used as standard. The CV of each sample triplicate was normally <10%. The ELISA was repeated three times for each sample, and the mean value was calculated. Repeated measurements of Wistar rats ($N = 2$) that received only corn oil gave a background of 0·04 fmol μg^{-1} DNA (SD 0·02) which was subtracted from each measurement. Estimated detection limit of the assay was 0·06 fmol B[*a*]P μg^{-1} DNA.

Albumin isolation from plasma

One ml saturated ammonium sulphate was added to 1 ml blood serum and the samples were incubated overnight at 4 °C. After centrifugation at ~750 *g* the precipitate was removed and 10 µl concentrated glacial acetic acid was added and the incubation continued overnight. After centrifugation (~750 *g*) the supernatant was removed and the precipitate washed in 4 ml acetone/ethylacetate (1 : 1) and the final precipitate left to dry at room temperature. Then the precipitate was solubilized in 900 µl 10 mM Tris–HCl/1·0 mM EDTA buffer, pH 8·0. The protein concentration was measured essentially by the method of Lowry (1951).

BPDE-I–DNA adduct determination in lung and liver tissue and in plasma albumin by HPLC analysis

The procedure was carried out basically as described by Rahn *et al.* (1982) and Rojas *et al.* (1994). DNA (1 mg) was incubated in HCl at a final concentration of 0·1 M at 90 °C for 4 h. The sample was applied on a primed Sep-pak C_{18} cartridge (Millipore, Milford, MA). The cartridge was washed with 5 ml water and eluted with 10 ml methanol. The eluate was evaporated to dryness with nitrogen gas at 40 °C and the residue dissolved in 400 µl 10% methanol. The plasma-albumin samples were incubated with 0·1 M HCl for 3 h at 90 °C. The 1 ml sample was diluted with 4 ml 10% methanol and purified on Sep-pak C_{18} as above. The sample was eluted with 5 ml methanol, evaporated to dryness, and dissolved in 500 µl 10% methanol. For the analysis, 200 µl of each sample was injected into the HPLC.

HPLC analysis was performed with a Nova-pak® C_{18} 4 µm, 3·9 × 150 mm column (Waters, Milford, MA) at 40 °C and flow 1·0 ml min^{-1}. The B[*a*]P-tetrols were separated by a methanol and water gradient (30% to 100% methanol in 40 min; then 100% methanol in 10 min followed by 30% methanol in 20 min). The analysis was carried out with a Waters 625 LC system (Waters, Milford, MA) equipped with a LC 240 fluorescence detector (Perkin-Elmer Ltd, Beaconsfield, UK). The excitation wavelength was 341 nm with emission at 381 nm. The detection limit was 0·01 fmol B[*a*]P-tetrol μg^{-1} DNA when quantitating 500 µg DNA per injection.

Results

We have measured the CYP1A1 activity by the EROD assay in rat liver with and without MC pre-treatment at several time points (figure 1). The single dose of B[*a*]P resulted in a transient increase in EROD activity. The EROD activity in liver-microsomes from the MC-treated animals was on average 2·4 times higher than in microsomes from the control group.

The B[*a*]P–DNA and the BPDE-I–DNA adduct levels in lung tissue reached a maximum around 7 days after a single administration of B[*a*]P both for the MC-induced rats and for the control rats (figures 2 and 3). The B[*a*]P–DNA adduct levels in liver measured with ELISA did not exhibit a prominent peak and the maximum level was found at 4 days (figure 2). By HPLC, liver BPDE-I–DNA adduct levels in MC-treated rats reached a maximum 1 day after the B[*a*]P injection and showed a transient reduction before another increase (figure 3). The BPDE-I–albumin adduct level reached a peak around 1 day after B[*a*]P-exposure (average 0·93 fmol μg^{-1} protein (SE 0·12) for MC-treated rats and 0·64 fmol μg^{-1} protein (SE 0·19) for control rats) and declined rapidly.

For comparison, the data shown in figures 2 and 3 are summarized in table 1 as area under the curve (AUC). As listed in table 1 the ELISA measurements gave consistently higher DNA-adduct levels than the HPLC measurements in both lung and liver tissue. From the immunoassay measurements, induction by MC prior to administration of B[*a*]P resulted in increased B[*a*]P–DNA adduct levels of 1·5 times in both lung and liver tissue. By HPLC, a negligible (1%) increase resulting from MC-induction could be seen on the BPDE-I–DNA adduct levels in lung in contrast to a 1·5 times increase in liver tissue. However, the very low adduct levels in liver measured by HPLC causes higher relative error. Higher levels of B[*a*]P–DNA adducts were found in liver than in lung when measured with immunoassay. When quantitating only BPDE-I–DNA by HPLC, higher adduct levels were found in lung than in liver.

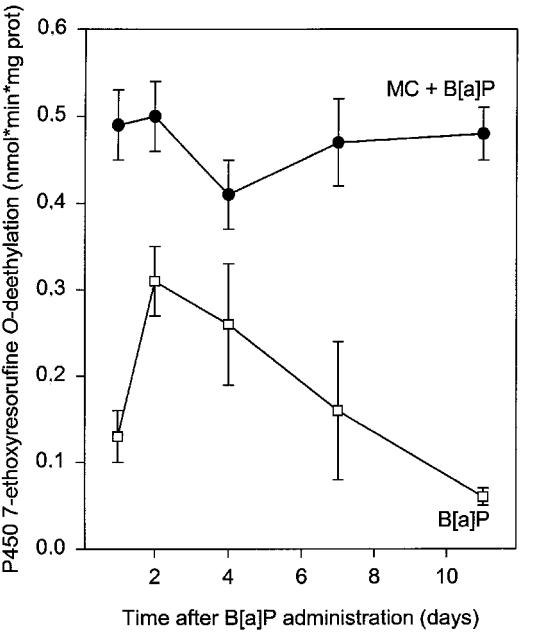

Figure 1. Ethoxyresorufin *O*-deethylation (EROD) activity measured in liver microsomes from rats exposed to methylcholanthrene and B[*a*]P and from control rats exposed to only B[*a*]P. Measurements at days 1, 2, 4, 7 and 11 after B[*a*]P administration. Each point represents the mean of six or four animals. Standard errors are shown with vertical bars.

The correlation between CYP1A1 activity in liver and B[*a*]P-adduct formation was calculated (table 2). Whereas there were no significant correlation between adduct levels in non-induced tissue and EROD activity measured in liver microsomes, increased B[*a*]P and BPDE-I–DNA and BPDE-I–albumin adduct levels were significantly correlated to increased EROD activity, except for BPDE-I–DNA adducts in liver. BPDE-I–albumin adduct levels were negatively correlated to increased EROD activity in liver (table 2).

The correlation coefficients between B[*a*]P–DNA adduct levels in lung and liver measured with both methods are shown in table 3. The correlation between BPDE-I–albumin adducts and B[*a*]P–DNA adducts in lung and liver tissue was not estimated due to large differences in half life between these adducts.

Discussion

In rodents, induction of CYP1A1 is usually associated with increased risk of PAH-induced mutagenesis and carcinogenesis (Nebert 1989). The EROD activity was increased 2·4 times in liver exposed to MC and B[*a*]P compared with rats exposed only to B[*a*]P and the CYP1A1 induction corresponded significantly to the 1·5 times increase of total B[*a*]P–DNA adducts in both lung and liver measured with immunoassay. However, we found only a small increase in the level of BPDE I–DNA adducts in lungs of MC-induced rats. This may partly be explained by the exposure to B[*a*]P which itself is an inductor of CYP1A1. Further, the elevated level of BPDE-I–DNA adducts in the liver of MC-treated rats was not significantly correlated to the increased liver CYP1A1 activity. In animal studies,

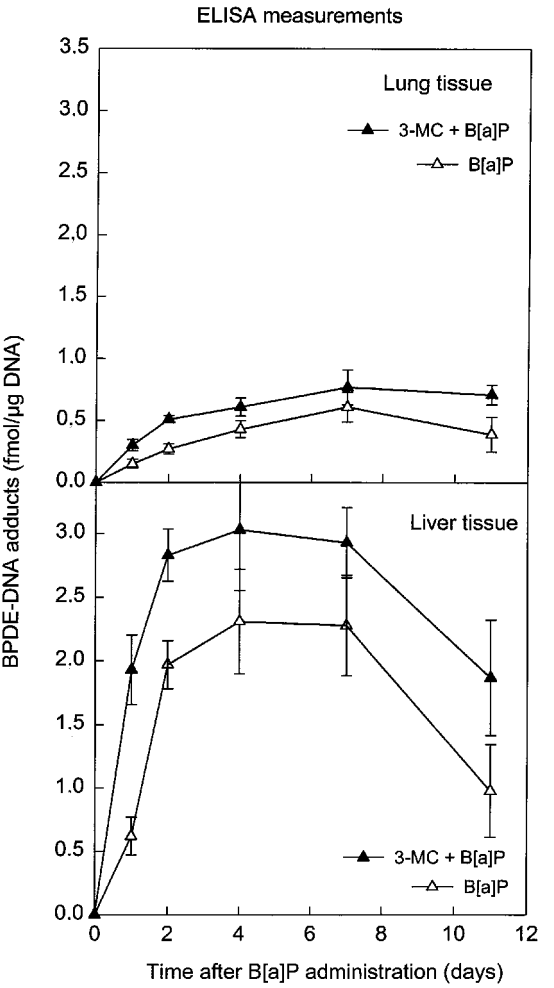

Figure 2. B[*a*]P–DNA adducts in lung and liver tissue from methylcholanthrene-induced rats and control rats, measured by immunoassay. Each point represents the mean of six or four animals (see table 1). Standard errors are shown with vertical bars.

although liver has the highest level of CYP1A1 activity, lung and skin tissue has been found to be more efficient in the metabolism of PAHs to its ultimate carcinogens, as reviewed by Hall and Grover (1990).

Several *in vitro* studies indirectly support our findings of increased B[*a*]P–DNA adduct formation in rat liver following MC-induction (Jones *et al.* 1983, Roggeband *et al.* 1993, Yang *et al.* 1997). On the other hand, results published by Bjelogrlic *et al.* (1993) showed that although liver microsomes from MC-treated mice catalysed the *in vitro* formation of BPDE–DNA adducts more efficiently than un-induced microsomes, MC exposure resulted in decreased amount of BPDE–DNA adducts *in vivo* in both lung and liver measured by synchronous fluorescence spectrophotometry. The authors suggested that the discrepancy may be due to lack of glutathione *S*-transferases (GST) *in vitro*, which are known to inhibit binding of B[*a*]P to DNA (Hesse *et al.* 1982, Bjelogrlic *et al.* 1993). An example of the protective effect by induction of the P450 enzyme system is given

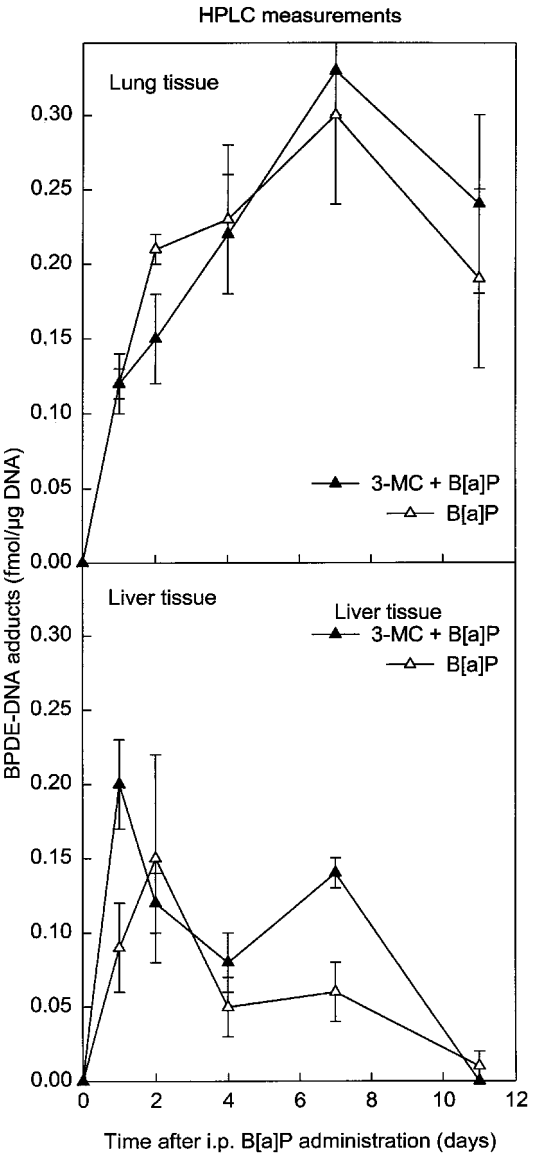

Figure 3. BPDE-I–DNA adducts in lung and liver tissue from methylcholanthrene-induced rats and control rats, measured by HPLC. Each point represents the mean of six or four animals. Standard errors are shown with vertical bars.

by Olsen *et al.* (1993) who observed that the level of 4-aminobiphenyl adducts in rat haemoglobin and in bladder and liver DNA were significantly lower in rats given phenobarbital prior to 4-aminobiphenyl. Because adduct levels reflect the balance between formation and detoxification of reactive metabolites, *in vivo* animals studies are the most relevant when studying the effect of P450 inducers on adduct formation.

Although the main site for B[*a*]P-carcinogenesis is the lung, the total B[*a*]P–DNA adduct levels as measured by immunoassay were more than three times higher in liver than in lung from both non-induced and MC-treated rats.

Table 1. Area under curve (AUC) for B[*a*]P- and BPDE-I adducts measured in lung- and liver-DNA and in plasma albumin after exposure to MC and B[*a*]P, and in control rats given corn oil and B[*a*]P .

	Immunoassay				HPLC			
Adduct site	MC-treated group	Control group	Increase	2-tail (*P*) significance	MC-treated group	Control group	Increase	2-tail (*P*) significance
Lung	7·0[a]	4·8	1·5	0·12[b]	2·3	2·3	1·0	0·91
Liver	23·8	15·4	1·5	0·02	0·6	0·4	1·5	0·21
Albumin	—	—			2·0	1·5	1·3	0·44

[a] Area under curve (AUC) calculated with the trapezoidal technique.
[b] Paired Student's *t*-test.
Quantitation by ELISA ($N = 52$) and HPLC ($N = 51$) at day 1,2,4,7 and 11 after B[*a*]P exposure.
DNA adducts are measured in fmol μg^{-1} DNA and albumin adducts in fmol μg^{-1} protein.

Table 2. Correlation (Spearman rank test on AUC[a] values) between cytochrome P450IA1 activity in liver microsomes and B[*a*]P-adduct formation in liver and lung tissue and in plasma albumin quantitated at five time points by immunoassay and an HPLC method.

	Immunoassay		HPLC	
	Control group	MC treated group	Control group	MC treated group
Lung	0·50[b] (0·391[c])	1·00 (>0·001)	0·50 (0·391)	0·90 (0·037)
Liver	0·80 (0·104)	0·90 (0·037)	–0·20 (0·747)	–0·70 (0·188)
Albumin			–0·10 (0·873)	–0·90 (0·037)

[a] Area under curve (AUC) calculated with the trapezoidal technique.
[b] Correlation coefficient (*r*).
[c] Significance (*P*).

Table 3. Correlation between B[*a*]P–DNA and BPDE-I adduct levels in rat tissues quantitated by immunoassay and an HPLC method.

Method and tissue	Correlation coefficient (*r*)	Number of samples (*N*)	Significance (*P*)
ELISA lung vs ELISA liver	0·58	52	< 0·001
ELISA lung vs HPLC lung	0·46	52	0·001
ELISA liver vs HPLC lung	0·47	52	< 0·001
ELISA lung vs HPLC liver	–0·02	51	0·913
ELISA liver vs HPLC liver	0·26	51	0·066
HPLC liver vs HPLC lung	–0·04	51	0·080

This observation corresponds with the ^{32}P-postlabelling data of Ross *et al.* (1990) who found higher adduct level in rat liver compared with lung after a single i.p. dose of B[*a*]P (100 mg kg^{-1}) when measuring all B[*a*]P adducts. By HPLC analysis we found a higher level of BPDE-I–DNA adducts in the target lung tissue than in liver from both induced and control rats. Similar results on B[*a*]P-exposed rats were observed *in vivo* by ^{32}P-postlabelling (Ross *et al.* 1990) and HPLC-analysis (Godschalk *et al.* 1997). In addition to the major adduct BPDE-I–dGuo, Ross *et al.* (1990, 1991) and Qu and Stacey (1996) detected two other major adducts found in liver and lung that were derived from B[*a*]P-*trans*-7,8-dihydrodiol and from 9-OH-B[*a*]P-4,5-epoxide, respectively. Theoretically it should be possible to quantitate these adducts by HPLC, however they may behave differently, both spectroscopically and with regard to their stability, from the BPDE-I–DNA

adducts precluding our finding of these adducts since we only found trace amounts of other adducts.

We found significant correlations between B[*a*]P–DNA adducts in lung and in liver measured with the same and different methods except from measurements by HPLC in liver. We would expect to find a relationship between these two adduct quantitation methods since the BPDE-I–DNA adduct is a major adduct in lung and liver and an adduct detected by immunoassay. However, in a study on human samples, Rojas *et al.* (1994) found no correlation between immunoassay and HPLC measurements of BPDE–DNA adducts.

The times to reach maximum adduct levels were similar for the MC-treated and the control rats, even though the CYP1A1 activity was higher for the induced rats compared with the control group. In our study, total B[*a*]P– and BPDE-I–DNA adduct levels reached a peak around 7 days after the single B[*a*]P administration in both induced and non-induced lung tissue measured by both methods, and at 1 and 4 days in liver, measured with HPLC and immunoassay, respectively. Similar findings are reported in rat by Ross *et al.* (1990) who observed a maximum level of BPDE-I–dGuo in lung, liver and in peripheral blood lymphocytes at 3–4 days after a single i.p. B[*a*]P dose. The low adduct level found in liver by the HPLC method is the main reason for the peculiar form of the time curve. The possible inconsistencies in these data are reflected in the correlations with HPLC liver DNA-adduct levels which are lower than the other comparisons shown in table 3. Both assays were found to be sensitive and the detection limits were similar for the two adduct quantitation methods.

Carcinogens bound to plasma proteins are likely to be proportional to binding to DNA. The BPDE-I–albumin adducts reached a maximum after 2 days and thereafter declined rapidly in accordance with the short half-life of rat albumin of 4 days (Skipper *et al.* 1984). The half-life of DNA adducts is mainly determined by DNA repair, and it is apparent that because of the differences in the half-life of BPDE-I adducts in albumin and DNA in this study a correlation analysis would not be meaningful as in chronic exposure as studied by Qu and Stacey (1996). Ross *et al.* (1990) estimated the half-life of total B[*a*]P–DNA adducts to be 15 days in liver and 22 days in lung after a single i.p. dose of B[*a*]P.

In summary, the results from this study show that MC-treatment of rats exposed to B[*a*]P results in a significant increase in B[*a*]P–DNA adduct formation *in vivo* in both lung and liver measured by immunoassay. Measurements of total B[*a*]P–DNA adducts by immunoassay gave higher adduct levels in liver than in lung, while the opposite was found when quantitating the BPDE-I–DNA adduct by the HPLC method.

Acknowledgements

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