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Comparative ^{32}P-postlabeling analysis of benzo[*a*]pyrene–DNA adducts formed *in vitro* upon activation of benzo[*a*]pyrene by human, rabbit and rodent liver microsomes*

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An interspecies comparison was made of the DNA-adducts formed *in vitro* upon incubation of rat liver DNA (RL-DNA) with benzo[*a*]pyrene (BP) in the presence of liver microsomes. Incubations were carried out with RL-DNA, BP (100 μM) and liver microsomes from hamsters, mice, rabbits, rats, 3-methylcholanthrene (3MC) pretreated rats and from humans. To analyse the adduct profiles, the ^{32}P-postlabeling technique with the nuclease P1-enhancement procedure was used. The total amount of adduct formed varied greatly with the species; also the number of adduct spots detected was different, ranging from 1 to 5. In all incubations the BP-N^2-deoxyguanosine adduct was formed. Relative to the total adduct level, the level of this adduct varied from 26% with rat, 54% with hamster, 56% with 3MC-pretreated rat, 58% with mouse and 75% with rabbit, to 100% with human liver microsomes. In human liver microsomes both the total amount of cytochrome P-450 per mg microsomal protein and the ethoxyresorufin *O*-deethylation (EROD) activity were low compared to that in animal liver microsomes. In microsomes from 3MC-pretreated rats the EROD activity was strongly induced. There was no correlation between EROD activity in non-induced microsomes and total adduct level. To compare BP–DNA adduct formation in human white blood cells (WBC) with that in RL-DNA, WBC were incubated with BP and 3MC-pretreated rat microsomes. The adduct profile in WBC-DNA differed from that observed after incubation of RL-DNA: the BP-N^2-deoxyguanosine adduct in WBC-DNA accounted for 97% of the total adduct level. It is concluded that the ^{32}P-postlabeling method is a suitable technique to investigate both qualitative and quantitative differences in BP–DNA adduct formation between species. Furthermore, the incubation of microsomes from the liver (or other sources) with a genotoxic agent and isolated DNA or cells can be a useful approach to study the formation and stability of reactive intermediates that are able to bind to DNA, also with respect to differences between species or tissue.

Introduction

Polycyclic aromatic hydrocarbons (PAH*) are a class of chemical carcinogens that are ubiquitous environmental pollutants (1). Exposure to PAH occurs via the respiratory tract, the digestive tract or the skin. To become carcinogenic, PAH have to be activated through metabolic conversion. PAH are metabolized mainly by the enzymes of the cytochrome P-450I family, present in specific cell types in various tissues. Metabolism of PAH has been elucidated in great detail through studies of benzo[*a*]pyrene (BP), the widely used model compound for PAH (2,3). The formation of epoxides is the initial step in the metabolism of unsubstituted PAH (4). The so-called 'bay-region' epoxides which contain a sterically hindered area, e.g. the region between C-10 and C-11 in BP, show the highest chemical reactivity (5). Epoxides can be hydrated to yield diols or they can isomerize to phenols. The majority of the PAH metabolites are detoxicated and either excreted as conjugates of glutathione via the urinary tract or as conjugates of glucuronic acid via the digestive and/or urinary tract (4). A small proportion of the reactive metabolites binds covalently to cellular macromolecules, such as DNA, and forms adducts (6–8). In the case of BP the (+)-*anti*-BP-7,8-diol-9,10-epoxide ((+)-*anti*-BPDE) is considered to be the ultimate carcinogen. In metabolism studies, liver microsomes are often used to achieve activation of PAH (9–12). With respect to the metabolism of PAH, it is known that both quantitative and qualitative differences exist between different rodent species (13).

The objective of the work presented here was to study the BP–DNA adduct formation *in vitro* after incubations of DNA with BP and rabbit, rodent or human liver microsomes, by use of the ^{32}P-postlabeling method (14–17). The sensitivity of this method and the specific adduct patterns obtained upon multidirectional TLC render it possible to study both quantitative and qualitative aspects of adduct formation. Species-specific biotransformation was studied by use of microsomes, obtained from hamster, rat, mouse, rabbit and human livers. Liver microsomes were chosen because the liver plays a central role in the mechanism of PAH carcinogenicity (18). Also, liver microsomes were isolated from rats that had been treated with 3-methylcholanthrene (3MC), a known P-450IA inducer in rats (19). Both the ethoxyresorufin *O*-deethylation [EROD, indicative of activity of P-450IA1 and P-450IA2, (20)] and the pentoxyresorufin *O*-depentylation [PROD, indicative of P-450IIB, (21)] activity of all microsome preparations were determined.

Furthermore, a comparison was made between the adducts formed upon incubation of DNA with BP and liver microsomes from 3MC-treated rats (3MC-microsomes) and those found upon incubation of human white blood cells (WBC) with BP and these microsomes.

Materials and methods

Chemicals and enzymes

Glucose-6-phosphate, NADP, glucose-6-phosphate dehydrogenase (G6PD), nuclease P1 and RNAse T1 were obtained from Boehringer, Mannheim, Germany.

*Abbreviations: PAH, polycyclic aromatic hydrocarbons; BPDE, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; BP, benzo[*a*]pyrene; (+)-*anti*-BPDE, (+)-*anti*-BP-7,8-diol-9,10-epoxide; 3MC, 3-methylcholanthrene; PROD, pentoxyresorufin *O*-depentylation; WBC, white blood cells; G6PD, glucose-6-phosphate dehydrogenase; EROD, ethoxyresorufin *O*-deethylation; RL-DNA, rat liver DNA; PEI, poly(ethylenimine).

Table I. Characteristics of microsome preparations from various species

Species	Protein[a]	Cytochrome P-450[b]	EROD[c]	PROD[c]
Hamster	15.2 ± 0.3	0.63 ± 0.04	41.0 ± 1.3	2.9 ± 0.1
Mouse	22.6 ± 0.4	0.73 ± 0.13	37.8 ± 0.6	5.5 ± 0.5
Rat (Wag/MBL)	23.7 ± 2.7	0.74 ± 0.16	NA	3.6 ± 0.1
Rat (Wistar)	22.5 ± 2.1	0.71 ± 0.02	34.2 ± 1.6	3.8 ± 0.3
Rat-3MC (Wistar)	18.8 ± 1.0	0.59 ± 0.04	120 ± 6.5	10.0 ± 0.0
Rabbit	17.5 ± 0.8	0.38 ± 0.12	23.5 ± 0.5	4.4 ± 0.7
Human (061285)	22.3 ± 1.6	0.13 ± 0.03	6.9 ± 0.8	0.2 ± 0.0
Human (071087)	16.7 ± 0.2	0.15 ± 0.00	10.1 ± 3.3	ND
Human (250589)	26.7 ± 3.4	0.15 ± 0.02	5.9 ± 0.4	ND
Human (130192)	20.0 ± 1.5	0.13 ± 0.01	7.3 ± 0.9	ND

[a]Microsomal protein content in mg per ml ± SD (n = 3).
[b]Total amount of cytochrome P-450 in nmol per mg protein ± SD (n = 3).
[c]Activity in pmol resorufin formed per min per mg protein ± SD (n = 3).
NA, not analysed.
ND, not detectable.

RNase T1 was heated at 80°C for 5 min to remove DNase activity. Micrococcal endonuclease, spleen phosphodiesterase, RNase A, BP and 3MC were obtained from Sigma, St Louis, MO. T4 polynucleotide kinase was purchased from Biolabs, Beverly, MA and proteinase K from Merck, Darmstadt, Germany. Racemic *anti*-BPDE was obtained from Chemsyn, Lenexa, KS. γ-[^{32}P]ATP was purchased from Amersham, Buckinghamshire, UK.

Preparation of microsomes

Livers (from young male animals that served as untreated controls in other experiments) were isolated from rats (Wag/MBL and Wistar), mice (BALB/c), rabbits (New Zealand White) and hamsters (Syrian Golden) and homogenized with a Potter Elvehjem homogenizer in 3 vol. of 0.1 M potassium phosphate buffer, pH 7.4 (1 g of liver in 3 ml of buffer). The liver suspension was centrifuged at 9000 *g* for 30 min in a cooled (4°C) Beckman centrifuge. Thereafter, the supernatant (S9) was centrifuged at 100 000 *g* for 90 min. The microsomal pellet was resuspended in 0.1 M potassium phosphate buffer, pH 7.4. Procedures for preparation of microsomes were identical for livers of all species.

To obtain 3MC-induced microsomes, male young adult Wistar rats were injected i.p. with 3MC in corn oil (40 mg 3MC/kg body wt) on three consecutive days. One day after the third injection, liver microsomes were prepared according to the protocol described above.

Human microsomes were obtained from livers of four individuals (all males). The liver tissue (recovered from two persons who died in a traffic accident and two persons who died of cerebral haemorrhage) had been kept frozen (−80°C) for 6 months to 7 years.

The cytochrome P-450 and protein content of the microsomes as well as the EROD and PROD activity were determined in triplicate according to the protocol described by Rutten *et al.* (22,23).

Isolation of rat liver DNA (RL-DNA)

Livers from untreated male rats were cut into pieces and homogenized in buffer A (250 mM sucrose, 100 mM EDTA, pH 7.4); per gram 9 ml was used (all vols are expressed per g liver as starting material). The homogenate was centrifuged (4000 *g*, 1 min, 4°C) and the pellet was resuspended in 9 ml buffer A. Subsequently, the solution was centrifuged (1000 *g*, 10 min, 4°C). The pellet was resuspended in 5 ml buffer B (250 mM sucrose, 25 mM EDTA and 1% Triton X-100, pH 7.4) and kept at 4°C for 30 min. Thereafter the chromatin was isolated by centrifugation (1000 *g*, 10 min, 4°C). Triton X-100 was removed by resuspending the chromatin in 5 ml buffer C (10 mM Tris−HCl pH 7.6, 25 mM EDTA), followed by centrifugation (1000 *g*, 10 min, 4°C). This last step was repeated once and finally the chromatin pellet was resuspended in 2.5 ml TEN buffer (20 mM Tris−HCl pH 7.6, 1 mM EDTA and 100 mM NaCl) and incubated overnight at 37°C with proteinase K (100 μg/ml) and 1% SDS. Extractions of DNA were carried out with equal vols of phenol, phenol-chloroform-isoamylalcohol (25:24:1, v/v/v) and chloroform-isoamylalcohol (24:1, v/v). The DNA was precipitated by addition of 0.1 vol of 3 M sodium acetate, pH 6.0 and 3 vols of ethanol at −20°C, washed with 70% ethanol at −20°C and dried *in vacuo*. Subsequently, the DNA was dissolved in 2.5 ml TE buffer (10 mM Tris−HCl pH 7.6, 1 mM EDTA) and treated serially with a mixture of RNAses A (50 μg/ml) and T1 (50 U/ml) for 90 min and proteinase K (100 μg/ml) for 60 min at 37°C. DNA was extracted, precipitated, washed and dried as described above. It was dissolved in MQ water and the concentration was estimated spectrophotometrically (1 mg of DNA per ml = 20 absorbance units at 260 nm). The $A^{260}/_{280}$ was 1.78.

Modification of RL-DNA

RL-DNA dissolved in Millipore-filtered (MQ) water (300 μl, 0.85 mg/ml) was mixed with 200 μl medium (100 mM HEPES pH 7.1, 1 mM $MgCl_2.6H_2O$, 10 mM glucose-6-phosphate, 1.6 mM NADP, 1 unit G6PD/ml) and 100 μl of a microsome suspension (protein content varied from 15.2 to 26.7 mg/ml in the different microsome preparations, see also Table I). BP, dissolved in DMSO, was added to a final concentration of 100 μM and the mixture was incubated for 90 min in a humidified incubator (37°C, 5% CO_2) according to the procedure described by Gorelick and Wogan (10). The final concentration of DMSO was 0.5% (v/v). After the incubation, DNA was extracted and purified as described above. It should be emphasized that despite the fact there is no RNA present in RL-DNA, incubations with microsomes can introduce RNA contamination which may influence the postlabeling assay. Contaminating RNA is, therefore, removed afterwards by a supplementary treatment of the modified RL-DNA with RNAses (see DNA isolation).

Isolation and BP treatment of human WBC

Blood was obtained from a healthy non-smoking female and collected in tubes containing EDTA. WBC were isolated at 4°C within 1 h after sampling. Red blood cells were lysed with a buffer containing 155 mM NH_4Cl, 10 mM $KHCO_3$ and 0.1 mM EDTA, pH 7.4 (3 ml of buffer per ml of blood). The WBC were then collected by centrifugation at 125 *g* and resuspended in RPMI medium without serum. The number of cells was determined in a haemocytometer (Burker, Germany) and adjusted to 5×10^6 cells per ml. The cell suspension was supplemented with glucose-6-phosphate (final concentration 5 mM), NADP (0.8 mM) and G6PD (0.4 units/ml). The microsome suspension was added (5% v/v). BP was dissolved in DMSO and added to a final concentration of 100 μM; the final concentration of DMSO was 0.5% (v/v). Subsequently, the mixture containing cells, microsomes and BP was transferred onto petri dishes. After 5 h of incubation in a humidified incubator (37°C, 5% CO_2) the cells were scraped off the petri dishes and washed with RPMI medium. Cells were collected by centrifugation at 125 *g* and frozen (−24°C). DNA from WBC was isolated by means of phenolic extractions. Typically, WBC (1×10^7)were suspended in 1 ml TEN buffer and treated further as described under Isolation of RL-DNA.

^{32}P-postlabeling analysis of BP−DNA adducts

The postlabeling assay was performed as described by Reddy and Randerath (15). DNA (5−15 μg) was digested with micrococcal nuclease (0.6 units) and spleen phosphodiesterase (0.012 units) and subsequently with nuclease P1 (3 units). The modified nucleotides were labelled by incubation with 37 MBq γ-[^{32}P]ATP (sp. act. > 5000 Ci/mmol) and 50 units T4-polynucleotide kinase for 30 min at 37°C.

The postlabeled mixtures were applied to 20 cm×20 cm poly-(ethylenimine) (PEI)-cellulose sheets (JT Baker, Phillipsburg, NJ). A paper wick was attached to the top of each TLC sheet; the sheets were developed overnight in 1 M sodium phosphate, pH 6.0 (D1). After two washes with water the sheets were developed in 8.5 M urea, 3 M lithium formate, pH 3.5 (D3) for 6.5 h and in 8.5 M urea, 0.8 M lithium chloride, 0.5 M Tris, pH 8.0 (D4), also for 6.5 h. To remove any remaining impurities, the sheets were developed overnight in 1.7 M sodium phosphate, pH 6.0 (D5, in the direction of D1). The adducts were localized by autoradiography on Kodak XAR-5 film with an intensifying screen. The spots on the PEI-cellulose sheets detected by autoradiography were cut out and the radioactivity was determined by liquid scintillation counting. The level of DNA adducts was calculated on the basis of the concurrent analysis of standard samples carrying known amounts of [^{3}H]BP-adducts. The exact amount of input DNA and the absence of RNA were determined by chromatography of an aliquot of the DNA digest on an FPLC-column (24).

Results

Cytochrome P-450 analysis of the microsomes

Table I summarizes the characteristics of the microsome preparations used. In human liver microsomes the amount of cytochrome P-450 per mg microsomal protein was significantly lower (P = 0.0003, Student's t-test) than in animal liver microsomes. Also the EROD activity was lower in human liver compared to animal liver microsomes. Strikingly, induction of the metabolic activity with 3MC in Wistar rats did not result in a higher amount of total cytochrome P-450 per mg microsomal protein. However, EROD and PROD were higher in the rat 3MC-microsomes compared to the untreated control.

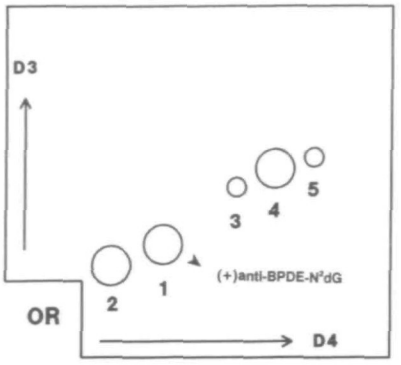

Fig. 1. Diagram of adduct profile, obtained upon postlabeling of BP-modified DNA. The position of adduct 1 corresponds with that of the adduct formed in the reaction between (+)-anti-BPDE and dG.

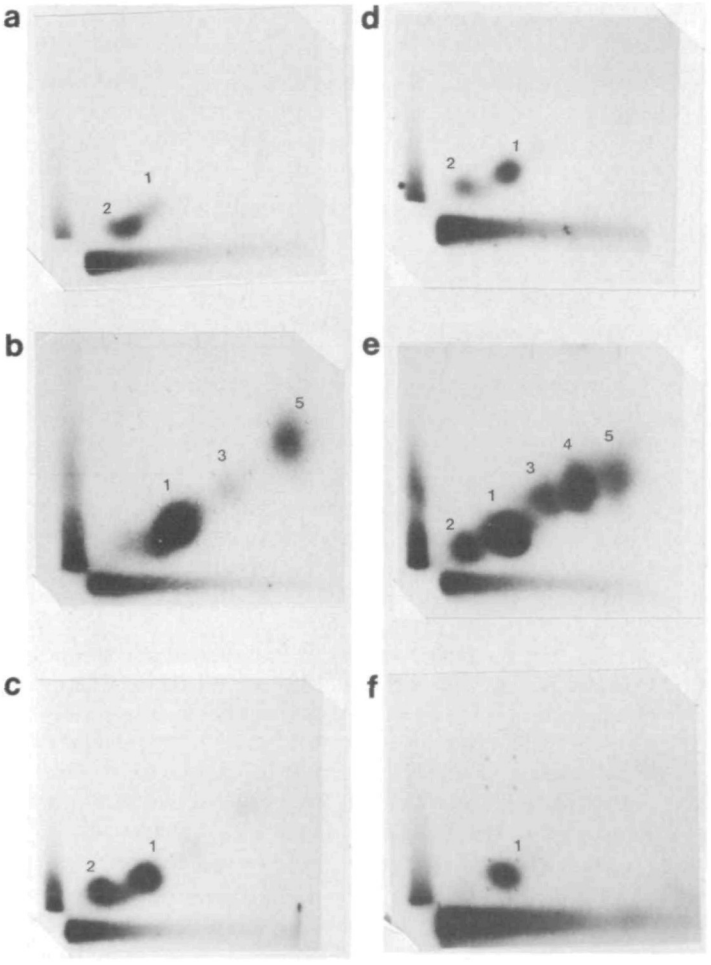

Fig. 2. Autoradiograms of BP-modified RL-DNA, obtained after incubation with both Wistar and Wag/MBL rat (**a**), rabbit (**b**), hamster (**c**), mouse (**d**), rat-3MC (**e**) and human (**f**) liver microsomes. Films (a–d) were exposed for 44 h, film (e) was exposed for 22 h and film (f) was exposed for 64 h at −70°C.

^{32}P-postlabeling analysis of BP–DNA adducts

Adducts formed in RL-DNA. Microsomes obtained from livers of various species were used to activate BP *in vitro*. RL-DNA was added as a target molecule. During the various BP experiments five different adduct spots were detected, of which the relative positions are indicated schematically in Figure 1.

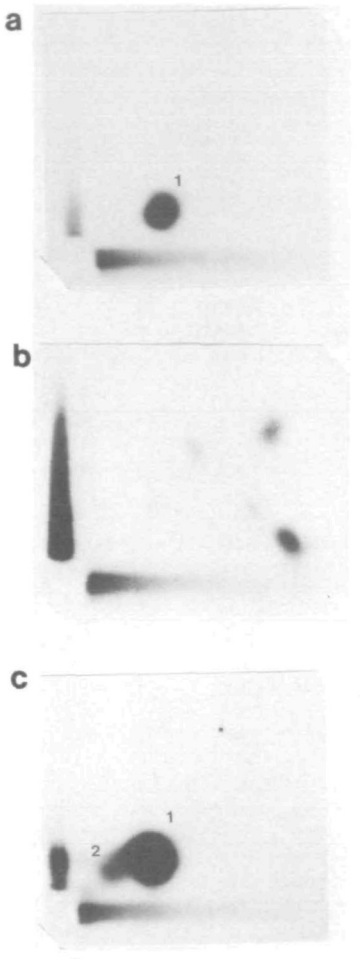

Fig. 3. Autoradiogram of (**a**) [^{3}H]BP-modified DNA at a level of 43 add/10^8n, (**b**) RL-DNA incubated with BP in the absence of microsomes and (**c**) BP-modified WBC-DNA, obtained after incubation of WBC with 3MC-induced rat-liver microsomes and BP. Films (a–c) were exposed for 22, 64 and 4 h respectively, at −70°C.

With liver microsomes from both the Wag/MBL rat (Figure 2a) and the Wistar rat (not shown) two adduct spots were observed. Adduct 1 co-chromatographed with the 3′5′-biphosphate of N^2-BP-deoxyguanosine (Figure 3a), the synthetic adduct obtained after incubation of (±)-*anti*-BPDE with RL-DNA (*trans* addition product of dG and (+)-*anti*-BPDE). The identity of adduct 2 and of other minor adducts observed in further experiments (see below), is not known. Rat-liver microsomes yielded a total adduct level per 1.5 mg of microsomal protein of 24.3 ± 10.2 adducts/10^8 nucleotides (add/10^8n) in the case of the Wag/MBL rat and 7.6 ± 0.6 add/10^8n for the Wistar rat. For both rat strains, adduct 2 was predominant (74%), compared with adduct 1 (26%). With rabbit-liver microsomes (Figure 2b) the total adduct level was 106 ± 37 add/10^8n, distributed over adduct 1 (75%), adduct 2 (15%) and adduct 5 (10%). There were no qualitative differences between the results of *in vitro* incubations with hamster liver microsomes (Figure 2c) and mouse liver microsomes (Figure 2d). In both cases the relative amounts of adducts 1 and 2 were comparable (54–59% of adduct 1 and 46–41% of adduct 2). With hamster liver microsomes the total adduct level was 158 ± 34 add/10^8n, compared to 22.0 ± 7.2 add/10^8n with mouse liver microsomes. For human liver microsomes, incubations with BP and RL-DNA (Figure 2f) resulted in only one spot, *viz.* adduct 1. The total adduct levels determined after incubation of the four different human liver-

Table II. BP–DNA adduct levels in add/10^8n after *in vitro* incubations of RL-DNA with BP in the presence of rodent, rabbit or human liver microsomes[a]

Species	Adduct 1[b]	Adduct 2	Adduct 3	Adduct 4	Adduct 5
Hamster	98.0 ± 0.3 (54%)[c]	82.6 ± 1.0 (46%)	ND	ND	ND
Hamster[d]	71.1 ± 19.2 (53%)	64.3 ± 16.0 (48%)	ND	ND	ND
Mouse	12.0 ± 5.2 (57%)	9.0 ± 4.7 (43%)	ND	ND	ND
Mouse[d]	14.0 ± 0.9 (61%)	9.0 ± 4.7 (39%)	ND	ND	ND
Rat (Wag/MBL)	7.4 ± 2.9 (26%)	21.2 ± 10.1 (74%)	ND	ND	ND
Rat[d] (Wag/MBL)	5.3 ± 0.2 (26%)	14.6 ± 0.6 (74%)	ND	ND	ND
Rat (Wistar)	2.6 ± 0.2 (32%)	5.4 ± 0.0 (68%)	ND	ND	ND
Rat[d] (Wistar)	2.2 ± 0.3 (30%)	5.0 ± 0.6 (70%)	ND	ND	ND
Rat-3MC (Wistar)	365 ± 97 (54%)	68.7 ± 6.5 (10%)	60.9 ± 3.4 (9%)	140 ± 24 (21%)	42.9 ± 0.8 (6%)
Rat-3MC[d] (Wistar)	433 ± 157 (58%)	83.6 ± 26.8 (11%)	59.8 ± 8.6 (8%)	126 ± 20 (17%)	42.4 ± 4.7 (6%)
Rabbit	105 ± 7 (75%)	ND	12.8 ± 0.3 (9%)	ND	22.8 ± 8.9 (16%)
Rabbit[d]	54.2 ± 6.4 (76%)	ND	8.1 ± 2.8 (11%)	ND	9.2 ± 1.1 (13%)
Human 061285	2.9 ± 0.2	ND	ND	ND	ND
Human 071087	2.9[e]	ND	ND	ND	ND
Human 250589	4.6 ± 0.7	ND	ND	ND	ND
Human 130192	2.2 ± 0.2	ND	ND	ND	ND

[a]Values are given as adduct levels/1.5 mg microsomal protein and as a mean of two independent postlabeling assays with range of values.
[b]See Figure 1 for adduct numbering.
[c]Relative percentage of one adduct.
[d]Independent second experiment with the same batch of microsomes.
[e]Only one postlabeling assay.
ND, not detectable.

microsome preparations with BP and RL-DNA, ranged from 2.9 to 4.6 add/10^8n. When corrected for the amount of cytochrome P-450 present during the microsomal incubations, the total BP–DNA adduct levels with human liver microsomes were in the same range as those found with rat and mouse liver microsomes.

When liver microsomes isolated from 3MC-pretreated rats were used in *in vitro* incubations with BP and RL-DNA (Figure 2e) an ~100-fold overall increase in BP–DNA adduct level was observed, compared to that found with microsomes from non-induced rats (711 ± 183 versus 7.6 ± 0.8 add/10^8n). Furthermore, the adduct profile showed some major differences compared to that obtained with non-induced rat-liver microsomes. The level of adduct 1 (~56%) was 399 ± 135 add/10^8n; the level of adduct 2, which was the major adduct observed in incubations with non-induced rat liver microsomes, was 76.2 ± 20.9 add/10^8n, i.e. only ~10% of the total adducts. A fairly large additional adduct spot appeared in the incubations with 3MC rat liver microsomes, *viz.* adduct 4 (133 ± 23 add/10^8n) which accounted for ~19%. Two minor spots, adducts 3 (60.4 ± 6.6 add/10^8n, 9%) and 5 (42.7 ± 3.4 add/10^8n, 6%) could also be detected. Adducts 3 and 4 may be derived, at least in part, from 3MC carried over during microsome preparation. The chemical may still be present in rat liver at 24 h after the third dose of 3MC. In a control experiment in which 3MC-induced rat-liver microsomes were incubated with RL-DNA in the absence of BP these two spots in positions 3 and 4 could be detected, although the levels of adducts 3 and 4 were ~3-fold lower in incubations without BP compared to incubations with BP (results not shown).

When untreated RL-DNA was analysed in the postlabeling assay, several spots could be detected (Figure 3b). The intensity of the individual spots would correspond to adduct levels in the range of 0.2 to 0.9 add/10^8n. In the postlabeling assay none of these adducts co-migrated with any of the BP–DNA adducts observed in other experiments.

Comparison of the EROD activity of non-induced microsomes (Table I) and the total BP–DNA adduct levels obtained with the same preparation (Table II) showed no correlation. Apparently, in livers the EROD activity is not predictive of the amount of BP–DNA adducts formed. However, 3MC-induced microsomes show an increased level of EROD and PROD activity and give rise to a considerable increase in DNA adduct induction.

Adducts formed in human WBC. When human WBC were incubated with BP (100 μM) in the presence of 3MC rat-liver microsomes (Figure 3c) adduct 1 was formed almost exclusively (97%); the DNA modification level was 903 ± 400 add/10^8n. The level of adduct 2 was 25.6 ± 12.8 add/10^8n (3%). The adducts 3, 4 and 5 could not be detected in incubations with human WBC and BP.

Adduct 1 could also be detected following incubations of WBC with BP (100 μM) in the absence of microsomes. However, the modification level was only 1.3 add/10^8n. The level of adduct 2, if formed, was too low to be detected.

Discussion

A comparative study was carried out on the *in vitro* activation of BP by liver microsomal preparations from various species. Purified rat-liver DNA was used as a target and the formation

of BP–DNA adducts was analysed by use of the ^{32}P-postlabeling method. It should be noted that microsomes often contain RNA. The presence of RNA may interfere with the adduct quantification in the postlabeling assay (24). Therefore, even though the initial DNA had been purified from RNA, following incubation with microsomes DNA should again be treated with RNAses.

The EROD activity is correlated with the ability of microsomes to metabolize planar PAH. Despite the fact that both the EROD activity and the total DNA-adduct level were lower with human liver microsomes compared to rabbit and rodent liver microsomes, there was no correlation between the EROD activity in non-induced microsomes and the total DNA-adduct level. Apparently, BP is metabolized by other P450 isoenzymes, besides P450IA1 and IA2. This is supported by the work of Nebert (19). Pretreatment of rats with 3MC resulted in a clear induction of the EROD activity along with an enhanced DNA-adduct formation. However, the total amount of cytochrome P-450 per mg of microsomal protein was not elevated. In a study of Wortelboer *et al.* (25) a similar observation was made: in male Wistar rats that were treated i.p. with β-naphthoflavone (an inducer of the EROD activity in rats) the EROD activity was increased 24-fold, compared with the control animals while in the same animals the total amount of P-450 was increased only 2.5-fold compared to the controls.

One of the adducts observed in the present study, adduct 2, has a low mobility in D3 and D4. Several studies with cultured cells from the rat provide evidence for the formation of DNA adducts derived from (±)-*syn*-BPDE (26–28). In order to find out whether adduct 2 is derived from (±)-*syn*-BPDE, RL-DNA was reacted with (±)-*syn*-BPDE (a generous gift of Dr A.Dipple). After purification, the (±)-*syn*-BPDE modified-DNA was analyzed by postlabeling. None of the (±)-*syn*-BPDE DNA adducts co-chromatographed with adduct 2 (not shown), so it can be concluded that this adduct is not derived from (±)-*syn*-BPDE. Another possibility may be that adduct 2 is derived from a metabolite of 9-OH-BP. By using 3MC-induced rat-liver microsomes Ashurst and Cohen (29) found that the major adduct formed in incubations with BP and DNA co-chromatographed with an adduct derived from a metabolite of 9-OH-BP. Ross *et al.* (30) exposed rats to 9-OH-BP i.p.; the major adduct formed in liver, lung and peripheral blood lymphocytes had a low mobility in both D3 and D4 compared to the reference (+)-*anti*-BPDE-dG adduct. The authors suggest that the major adduct is derived from the 4,5-epoxide of 9-OH-BP. The chromatographic behaviour of this adduct in their experiments is comparable to that of our adduct 2. Therefore, adduct 2 may be derived from the 9-OH-4,5-epoxide of BP. Experiments are ongoing to confirm the identity of this adduct. The reason that in our incubations of 3MC-induced rat-liver microsomes with BP and DNA adduct 2 was not a major adduct may be related to the level of epoxide hydrolase activity, which can shift metabolism towards diol-epoxide formation. Bodell *et al.* (9), who also used 3MC-induced rat-liver microsomes in *in vitro* incubations with BP and DNA, detected two major spots, of which one (55%) was identified as (+)-*anti*-BPDE-dG.

Incubations of human liver microsomes with BP and RL-DNA resulted in relatively low adduct levels, compared to the incubations with animal liver microsomes. This can be explained by a rather low content of cytochrome P-450 per mg microsomal protein in human liver microsomes. However, the total BP–DNA adduct levels were in the same range as those observed with rat and mouse liver microsomes when adduct levels were corrected for the amount of cytochrome P-450 present during the incubations. The biotransformation capacity of the human microsomes may have been reduced upon storage of the human liver tissue (32,33). However, in the relatively fresh human liver microsomes (stored for 6 months) the adduct level was in the same range as found for the microsomes that had been stored for several years. Furthermore, there were no qualitative differences in adduct patterns between the different human liver microsome preparations. A more reliable comparison, however, can only be made when a fresh human liver specimen becomes available. In contrast to the animal liver microsomes, the use of human liver microsomes resulted in only one adduct spot, *viz.* adduct 1. In the study by Moore *et al.* (28), in which human mammary epithelial cells were exposed to BP (2 μM), also mainly the (+)-*anti*-BPDE-deoxyguanosine adduct was formed.

Human WBC are able to metabolize PAH to a relatively low extent (34). In our experiments, exposure of WBC to BP (100 μM) without an additional metabolic activation system yielded a total adduct level of ~1.3 add/10^8n. The study reported by Gupta *et al.* (34) showed several adduct spots in incubations of WBC with 30 μM BP, whereas we observed just one adduct, *viz.* the (+)-*anti*-BPDE-dG (adduct 1). The main adduct (62%) in their study showed the same characteristics as our adduct 1. Generally, the total adduct level in their experiments was also higher (2.6–48.0 add/10^8n; 12 individuals) compared to our result. It should be pointed out that we determined the capacity to metabolize BP of WBC from only one individual. Also, interlaboratory variation in the postlabeling technique makes it rather difficult to make a quantitative comparison between results of different studies (35).

The use of 3MC-induced rat-liver microsomes in the incubations of WBC with BP gave rise to a considerable increase in WBC-DNA adduct formation. Two adduct spots were observed, of which adduct 1 accounted for 97%. In RL-DNA, 3MC-microsomes induced 5 different adducts. Adducts 3 and 4, which are probably derived from 3MC itself, at least in part, were not found in DNA of WBC. This might be related to the stability of the 3MC-metabolites. Furthermore, in the WBC incubations the ratio between adduct 1 and 2 changed in favor of adduct 1, compared to incubations with RL-DNA. This change in adduct profile may reflect the stability of reactive metabolites, the attainability of DNA, and/or the presence of cellular proteins and enzymes.

The results presented in this paper demonstrate that the postlabeling assay is a very suitable method to study and compare various biological systems with respect to their capability to bio-activate PAH into products that can react with DNA. This approach not only allows the study of the overall capacity of such systems to convert PAH into DNA-adduct-forming compounds, it also provides insight into the differences in the specificity with which the systems perform these conversions. Moreover, a useful indication can be obtained as to the stability of the products of the bio-activation, by comparing the adducts formed during an incubation of the system with the substance under investigation together with purified DNA, with those resulting from an identical incubation with DNA inside cells.

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