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The K-Region *trans*-8,9-Diol Does Not Significantly Contribute as an Intermediate in the Metabolic Activation of Dibenzo[*a,l*]pyrene to DNA-binding Metabolites by Human Cytochrome P450 1A1 or 1B1[1]

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ABSTRACT

Metabolic activation of the K-region *trans*-8,9-diol of the highly carcinogenic hexacyclic aromatic hydrocarbon dibenzo[*a,l*]pyrene (DB[*a,l*]P) by human cytochrome P-450 (P450) 1A1 and 1B1 was investigated in Chinese hamster V79 cell lines expressing human P450 1A1 or 1B1. P450 1A1 and 1B1 are the major P450s involved in metabolic activation of polycyclic aromatic hydrocarbons in human cells. The major DNA adducts formed by metabolism of DB[*a,l*]P in cultures expressing P450 1A1 or 1B1 resulted mainly from the fjord region (−)-*anti*-DB[*a,l*]P-11,12-diol 13,14-epoxide [(−)-*anti*-DB[*a,l*]PDE] and, to a lesser extent, (+)-*syn*-DB[*a,l*]PDE. In V79 cells expressing human P450 1A1, high amounts of as yet unidentified highly polar DNA adducts are formed in addition to the DNA adducts derived from DB[*a,l*]PDEs. Human P450 1A1 has been found to metabolize DB[*a,l*]P on its K-region to the *trans*-8,9-diol, and it has been proposed that the DNA binding of the parent compound in P450 1A1-expressing tissues may be partially mediated by activation of the K-region *trans*-8,9-diol to form bis-diol epoxides. V79 cells expressing human P450 1A1 or 1B1 formed only low amounts of DNA adducts after treatment with high doses of the K-region *trans*-8,9-diol. None of the adducts formed were identical to the main adducts formed in the same cell lines by metabolic activation of DB[*a,l*]P or (−)-DB[*a,l*]P-*trans*-11,12-diol. These results demonstrate that the K-region *trans*-8,9-diol does not significantly contribute to the genotoxicity of the very potent carcinogen DB[*a,l*]P in human cells or tissues expressing P450 1A1 or 1B1.

INTRODUCTION

The environmental pollutant DB[*a,l*]P[3] has been identified as the most potent carcinogenic PAH in mouse skin or in rat mammary gland (1–4). Studies on metabolic transformation and DNA binding of this hexacyclic hydrocarbon (Fig. 1) revealed that activation in human cells occurs predominantly on the fjord region to yield the diastereomeric (+)-*syn*- and (−)-*anti*-DB[*a,l*]PDE, with (*S,R,S,R*) and (*R,S,S,R*) configurations, respectively (5, 6). Incubation of the metabolic precursors of these diol epoxides, the (+)-(11*S*,12*S*)- and (−)-(11*R*,12*R*)-diols (Fig. 1), respectively, with MCF-7 cells (7) or microsomes of rodents pretreated with Aroclor 1254 (8) produced high levels of DNA binding and mutation induction only with the (−)-*trans*-11,12-diol, which was very efficiently converted to (−)-*anti*-DB[*a,l*]PDE. High tumor induction *in vivo* on mouse skin was observed after treatment with the (−)-*trans*-11,12-diol, whereas tumor induction was low after exposure to the (+)-*trans*-11,12-diol (9).

Investigation of the DNA binding of DB[*a,l*]P, catalyzed by individual P450 enzymes expressed in Chinese hamster V79 cells, revealed that human P450 1B1 almost exclusively catalyzes the formation of (−)-*anti*-DB[*a,l*]PDE through intermediate production of (−)-*trans*-11,12-diol (10). After treatment with high doses of the parent PAH, only small amounts of (+)-*syn*-DB[*a,l*]PDE-derived DNA adducts were detectable. The pattern of DB[*a,l*]P-DNA adducts found in V79 cells expressing human P450 1B1 was similar to that found in human mammary carcinoma MCF-7 cells (10). Human P450 1A1-expressing V79 cells metabolically converted DB[*a,l*]P not only to (+)-*syn*- and (−)-*anti*-DB[*a,l*]PDE but also to more polar metabolites, which formed several as yet unidentified DNA adducts (10). Human recombinant P450 1A1-expressing microsomes produced in human hepatoma Hep-G2 cells (11) or liver microsomes of rats after induction of P450 1A1 by MC (12) demonstrated that this P450 metabolizes DB[*a,l*]P to both the *trans*-11,12-diol and the K-region *trans*-8,9-diol (Fig. 1) in both species. Studies with β-naphthoflavone- and MC-induced rat liver microsomes or commercially available recombinant human P450 1A1 microsomes have recently shown that further metabolism of the K-region *trans*-8,9-diol by P450 1A1 results in the formation of several bis-diols containing an additional *trans*-diol group at the 11–12 or 13–14 position (13). DNA binding studies with DB[*a,l*]P and the recombinant human P450 1A1 microsomal system suggested that the K-region *trans*-8,9-diol was involved in the formation of a substantial amount of one highly polar DNA adduct (13).

To investigate the contribution of the K-region *trans*-8,9-diol to the total DNA binding of DB[*a,l*]P, we treated V79 cells stably expressing human P450 1A1 or 1B1 with DB[*a,l*]P, DB[*a,l*]P-*trans*-8,9-, and DB[*a,l*]P-*trans*-11,12-diols (Fig. 1). Total DNA binding was measured by ^{33}P-postlabeling and the DNA adduct patterns observed in HPLC analysis were compared to determine the role of bis-diol formation in metabolic activation of DB[*a,l*]P by human P450s.

MATERIALS AND METHODS

Chemicals. DB[*a,l*]P was purchased from Chemsyn Science Laboratories (Lenexa, KS). Synthesis of enantiomerically pure (+)- and (−)-*trans*-11,12-diols was described previously (8). Synthesis of racemic and enantiomeric K-region *trans*-8,9-diols was described separately (14).

Cell Culture, Treatment, and DNA Isolation. Chinese hamster V79 fibroblasts expressing human P450 1A1 (15) and human P450 1B1 (10) were cultivated in DMEM, supplemented with 4.5 mg/ml glucose, 1 mM sodium pyruvate, 4 mM glutamine, 10% FCS, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37°C, 7% CO_2, and 90% saturated atmospheric humidity, as described previously (10). The human P450-expressing V79 clones (1B1/4 and 1A1) were previously characterized for ethoxyresorufin *O*-deethylase activity (10). Cells were cultivated in the presence of 500 μg/ml geneticin sulfate, which was not present during treatment. Prior to treatment (24 h), cells were seeded at a density of ~1×10^6 cells per 75-cm^2 cell culture flask and grown in a total volume of 30 ml of medium. Then, 30 μl of a DMSO solution of DB[*a,l*]P or its racemic or enantiomeric *trans*-8,9- and *trans*-11,12-diols were added. After an incubation period of 6 or 24 h, cells were harvested, and DNA was isolated as described (10).

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[3] The abbreviations used are: DB[*a, l*]P, dibenzo[*a,l*]pyrene; PAH, polycyclic aromatic hydrocarbon; DB[*a,l*]PDE, dibenzo[*a,l*]pyrene-11,12-diol 13,14-epoxide; P450, cytochrome P-450; MC, 3-methylcholanthrene; HPLC, high-performance liquid chromatography; DB[*a,h*]A, dibenzo[*a,h*]anthracene; DB[*a,j*]A, dibenzo[*a,j*]anthracene.

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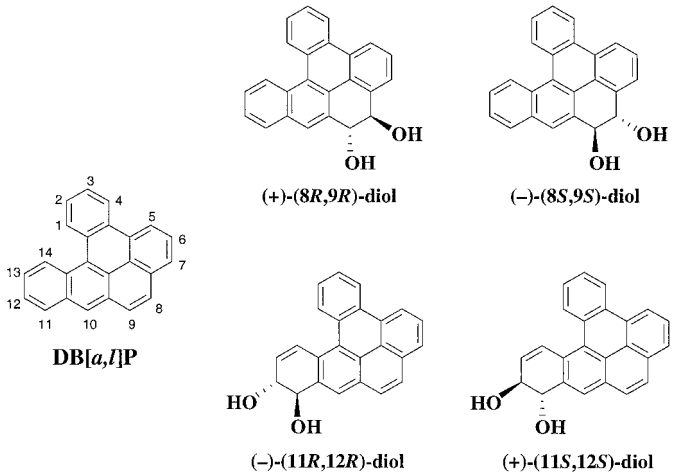

Fig. 1. Structures of the parent hydrocarbon DB[*a,l*]P and the enantiomeric *trans*-8,9- and *trans*-11,12-diols.

^{33}P-Postlabeling Analysis. DNA adducts were ^{33}P-postlabeled according to the protocol described previously (6). Briefly, 10 μg of DNA isolated from cells after treatment with DB[*a,l*]P or its *trans*-diols were digested with nuclease P1 and prostatic acid phosphatase, postlabeled with [γ-^{33}P]ATP (2000 Ci/mmol; NEN, Boston, MA), cleaved to adducted mononucleotides with snake venom phosphodiesterase I, and prepurified with a Sep-Pak C_{18} cartridge (6). The prepurification step on the Sep-Pak C_{18} cartridge (Waters, Milford, MA) was modified to ensure that no highly polar DNA adducts would be lost during the washing procedure. Prior to application of the sample, the cartridge was conditioned with methanol (10 ml), distilled water (10 ml), and loading buffer (10 ml; 0.5 M potassium phosphate, pH 6.0). The samples were diluted in loading buffer and loaded on the cartridge. The cartridge was washed three times with distilled water (10 ml). The ^{33}P-labeled DNA adducts were then eluted from the cartridge with 3 ml of 5% basic methanol (5% ammonia hydroxide-95% methanol), and the amount of radioactivity in the samples was determined by scintillation counting. An appropriate aliquot was then subjected to HPLC analysis.

Separation of DNA adducts by HPLC (Beckman HPLC system equipped with two model 110B pumps and a model 420 controller; Beckman Instruments Inc., St. Louis, MO) was carried out using a C_{18} reverse-phase column (5-μm Ultrasphere ODS, 4.6 $\times$ 250 mm; Beckman Instruments). The solvent system consisted of 0.1 M ammonium phosphate buffer (pH 5.5; solvent A) and 10% acetonitrile-90% methanol (solvent B) at a flow rate of 1 ml/min. Improved resolution of the highly polar DNA adducts was attained by altering the gradient used previously (6) to a more polar gradient. The gradient for elution of the adducts was as follows: 20–44% solvent B over 20 min; 44–49% solvent B over 40 min; and 49–65% solvent B over 60 min. The radiolabeled nucleotides were detected by an on-line radioisotope flow-detector (Packard Instruments, Downers Grove, IL), and the level of DNA binding was calculated based on labeling of a [^{3}H]benzo[*a*]pyrene-7,8-diol 9,10-epoxide-DNA standard (16).

RESULTS

DNA Binding of DB[*a,l*]P and DB[*a,l*]P-*trans*-8,9- and -*trans*-11,12-Diols in V79 Cells Expressing Human P450 1A1. The HPLC profiles of ^{33}P-labeled DNA adducts isolated from DNA of P450 1A1-expressing V79 cells 6 h after treatment with 1 μM DB[*a,l*]P or 0.05 μM (–)-DB[*a,l*]P-*trans*-11,12-diol are shown in Fig. 2. As observed previously (10), both substrates were converted to intermediates that formed several highly polar DNA adducts in addition to the fjord region DB[*a,l*]PDE-DNA adducts (Fig. 2). The polar DNA adducts eluted between 20 and 50 min (Fig. 2, *peaks 1–3*), whereas the DB[*a,l*]PDE-DNA adducts eluted between 70 and 120 min (Fig. 2, *A* and *B*, respectively) under the conditions used. The altered elution gradient that started with 20% solvent B increased the resolution of the highly polar DNA adducts compared with the previous gradient that started at 44% solvent B (10), facilitating analysis of individual polar adducts. Comparison of the elution profiles of the DNA adducts obtained after treatment with DB[*a,l*]P and (–)-DB[*a,l*]P-*trans*-11,12-diol (Fig. 2, *A* and *B*, respectively) indicates that the major polar DNA adducts derived from the parent hydrocarbon were the same as those derived from its (–)-*trans*-11,12-diol (Fig. 2, *peaks 1–3*). Small amounts of additional polar DNA adducts derived from DB[*a,l*]P eluted shortly after the major peaks (Fig. 2*A*, *peaks 2* and *3*). Calculation of the relative amounts of polar DNA adducts and DB[*a,l*]PDE-DNA adducts 6 h after treatment with both substrates revealed that the polar adducts accounted for ~32 and 65% of the total DNA binding of DB[*a,l*]P and (–)-DB[*a,l*]P-*trans*-11,12-diol, respectively (Table 1).

Incubation of V79 cells expressing human P450 1A1 with increasing doses of racemic *trans*-8,9-diol of DB[*a,l*]P resulted in the formation of three DNA adducts with retention times between 30 and 65 min (Fig. 2*C*, *peaks I–III*). Calculation of the total DNA binding revealed that only low levels of adducts were formed after treatment with 2.5–10 μM (±)-*trans*-8,9-diol (Table 1). The level of binding was even lower than that observed in cells treated with the (+)-*trans*-11,12-diol, which had previously been demonstrated to be a poor substrate for activation by human P450 1A1 (Table 1; Ref. 10).

A cochromatogram of the DNA adducts obtained in V79 cells expressing human P450 1A1 exposed to the (–)-*trans*-11,12-diol of DB[*a,l*]P with those obtained after treatment with the (±)-*trans*-8,9-diol is shown in Fig. 2*D*. This experiment demonstrated that the highly polar DNA adduct formed in the greatest amount from DB[*a,l*]P and (–)-DB[*a,l*]P-*trans*-11,12-diol (Fig. 2, *peak 2*) is not identical to the major DNA adduct derived from the K-region *trans*-8,9-diol (Fig. 2, *peak I*). Both DNA adducts have similar polarity and HPLC retention behavior, but they did not coelute (Fig. 2*D*). The HPLC profile of the DNA adducts obtained after treatment of human P450 1A1-expressing cells with 5 μM (+)-*trans*-11,12-diol is shown in Fig. 3*A*. This substrate was converted almost exclusively to (+)-*syn*-DB[*a,l*]PDE-DNA adducts eluting between 70 and 120 min. No highly polar DNA adducts that elute in the region of the (±)-*trans*-8,9-diol-derived DNA adducts I–III were formed.

DNA adduct profiles obtained 24 h after incubation of human P450 1A1-expressing V79 cells with individual enantiomers of DB[*a,l*]P-*trans*-8,9-diol (Fig. 1) at 2.5 μM are shown in Fig. 3 (*B* and *C*). Comparison with the adduct pattern derived from the racemic *trans*-8,9-diol (Fig. 2*C*) demonstrates that the DNA adduct I was derived from the (+)-*trans*-8,9-diol with *R,R*-configuration (Fig. 3*B*, *peak I*). In contrast, the second largest DNA adduct was derived from the (–)-*trans*-8,9-diol with *S,S*-configuration (Fig. 3*C*, *peak III*).

DNA Binding of DB[*a,l*]P and DB[*a,l*]P-*trans*-8,9- and -*trans*-11,12-Diols in V79 Cells Expressing Human P450 1B1. The HPLC profile of ^{33}P-labeled DNA adducts isolated from P450 1B1-expressing V79 cells 6 h after treatment with 0.05 μM (–)-*trans*-11,12-diol of DB[*a,l*]P is shown in Fig. 4*A*. The HPLC profile obtained after treatment with 1 μM DB[*a,l*]P was essentially identical (data not shown). As observed previously (10), DB[*a,l*]P and (–)-DB[*a,l*]P-*trans*-11,12-diol are metabolically activated by human P450 1B1 almost exclusively to the fjord region DB[*a,l*]PDEs. The major DB[*a,l*]PDE-DNA adducts eluted with retention times between 70 and 120 min (Fig. 4*A*), and they accounted for ~90% of all adducts formed after exposure to DB[*a,l*]P or (–)-DB[*a,l*]P-*trans*-11,12-diol (Table 1). Only small amounts of more polar DNA adducts were found in human P450 1B1-expressing cells. Expansion of the radioacitvity scale of the (–)-*trans*-11,12-diol-derived HPLC adduct profile between 10 and 70 min demonstrates that small amounts of several polar adducts (Fig. 4*A*′, *peaks 1–5*) were formed. These adducts together accounted for only ~10% of the total DNA binding

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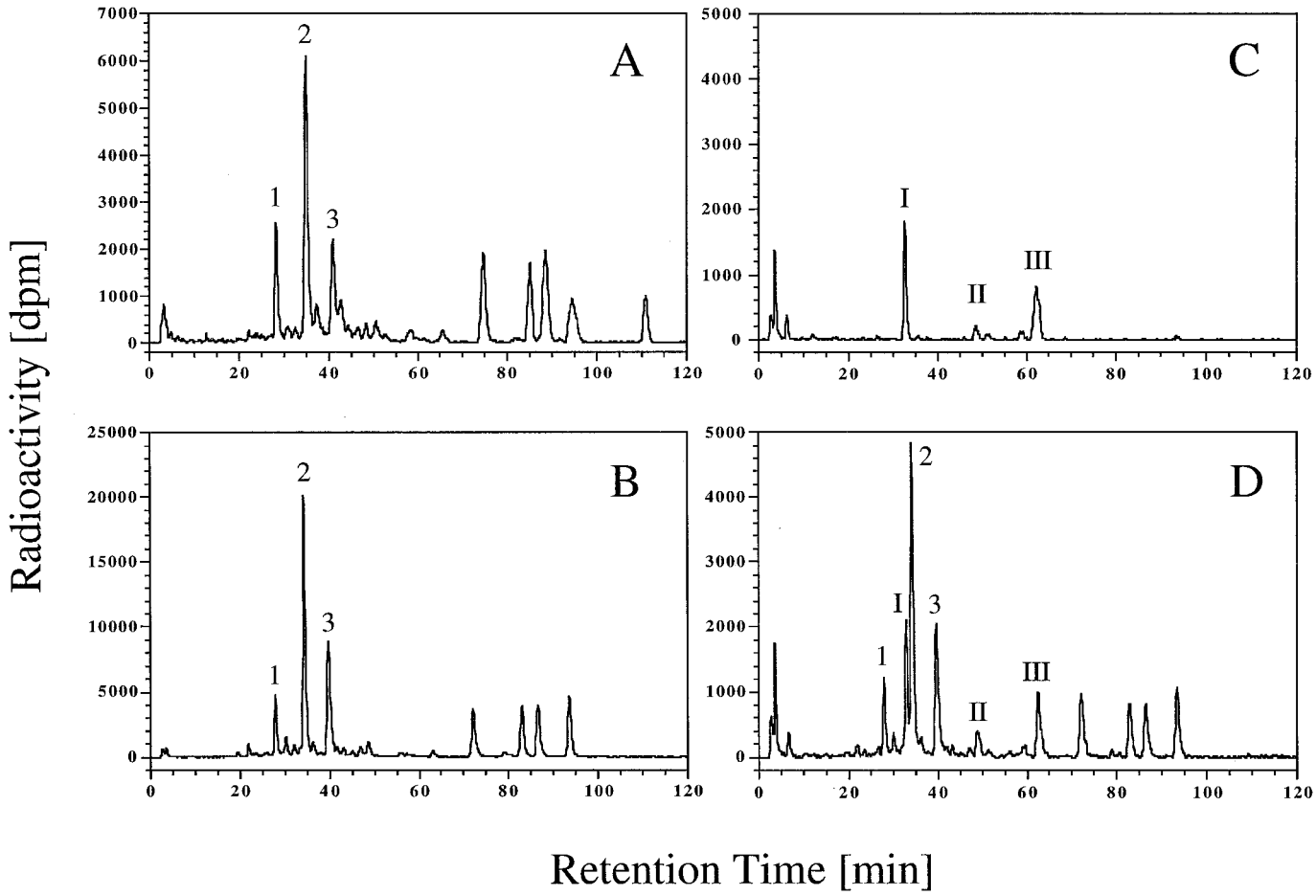

Fig. 2. HPLC elution profiles of ^{33}P-labeled DNA adducts formed in V79 Chinese hamster cells stably expressing human P450 1A1 6 h after treatment with 1 μM DB[*a,l*]P (*A*) or 0.05 μM (−)-*trans*-11,12-diol (*B*) and 24 h after treatment with 5 μM (±)-*trans*-8,9-diol (*C*). *Peaks 1–3*, highly polar unidentified (−)-*trans*-11,12-diol-derived DNA adducts eluting between 20 and 50 min; *peaks I–III*, (±)-*trans*-8,9-diol-derived DNA adducts. The HPLC elution profile of a mixture of aliquots of samples *B* and *C* (in a ratio of 1:4) is shown in *D*. HPLC conditions are described in "Materials and Methods."

of DB[*a,l*]P or (−)-DB[*a,l*]P-*trans*-11,12-diol catalyzed by human P450 1B1 (Table 1).

Incubation of human P450 1B1-expressing V79 cells with increasing doses of the racemic *trans*-8,9-diol of DB[*a,l*]P resulted in the formation of three different DNA adducts with retention times between 30 and 65 min (Fig. 4*B*, *peaks I–III*). Only low levels of DNA adducts were formed after treatment with 2.5–10 μM (±)-*trans*-8,9-diol (Table 1). The DNA binding of the (±)-*trans*-8,9-diol was in the same range as that observed in human P450 1A1-expressing cells and was again even lower than that observed after treatment with (+)-*trans*-11,12-diol (Table 1).

A cochromatogram of the DNA adducts obtained in V79 cells expressing human P450 1B1 exposed to the (−)-*trans*-11,12-diol of DB[*a,l*]P with those obtained after treatment with the (±)-*trans*-8,9-diol is shown in Fig. 4*C*. The results demonstrate that the major polar DNA adducts derived from (−)-DB[*a,l*]P-*trans*-11,12-diol (*peaks 1–4*) are not identical to the major DNA adducts I and II derived from the K-region *trans*-8,9-diol. However, one polar DNA adduct derived from the (−)-*trans*-11,12-diol of DB[*a,l*]P (*peak 5*) coeluted with the *trans*-8,9-diol-derived DNA adduct III (Fig. 4*C*). This polar DNA adduct accounted for ~3–4% of the total DNA adducts formed in human P450 1B1-expressing cells 6 h after treatment with DB[*a,l*]P or (−)-DB[*a,l*]P-*trans*-11,12-diol. The HPLC profiles of the DNA adducts obtained after treatment of human P450 1B1-expressing cells with high doses of (+)-*trans*-11,12-diol consisted almost exclusively of (+)-*syn*-DB[*a,l*]PDE-DNA adducts eluting between 70 and 120 min (data not shown). No polar DNA adducts that elute in the region of the (±)-*trans*-8,9-diol-derived adducts were detected.

Comparison of *trans*-8,9-Diol-derived DNA Adducts Formed in V79 Cells Expressing Human P450 1A1 and 1B1. Both P450 enzymes metabolized the K-region *trans*-8,9-diol of DB[*a,l*]P to intermediates that formed three DNA adduct peaks (Figs. 2*C* and 4*B*). The cochromatogram of the adducts obtained from both cell lines indicated that the major *trans*-8,9-diol-derived DNA adducts formed

Table 1 *Total DNA adducts in V79 Chinese hamster cells stably expressing P450 1A1 or P450 1B1 6 or 24 h after exposure to DB[a,l]P, (−)-trans-11,12-diol, (+)-trans-11,12-diol, or (±)-trans-8,9-diol*[a]

Concentration of compound (μM)	Human P450 1A1		Human P450 1B1	
	6 h	24 h	6 h	24 h
DB[*a,l*]P				
1.0	145 (98)	321 (195)	242 (212)	345 (317)
(−)-11,12-diol				
0.05	408 (141)	Not tested	443 (398)	Not tested
(+)-11,12-diol				
2.5			8.0 (7.6)	8.6 (7.7)
5.0	Not tested	34 (32)	7.6 (7.1)	12 (7.8)
(±)-8,9-diol[b]				
2.5	0.9 ± 0.5	4.0 ± 1.4	1.3 ± 0.2	3.1 ± 1.2
5.0	5.3 ± 2.8	6.2 ± 3.5	4.8 ± 1.9	4.0 ± 0.8
10.0	6.0 ± 2.2	15.1 ± 4.0	6.2 ± 3.8	8.5 ± 2.7

[a] All data are pmol adducts/mg DNA. Values represent total adducts present; values in parenthesis represent adducts of DB[*a,l*]PDE.

[b] Values represent the mean ± SD of three independent experiments.

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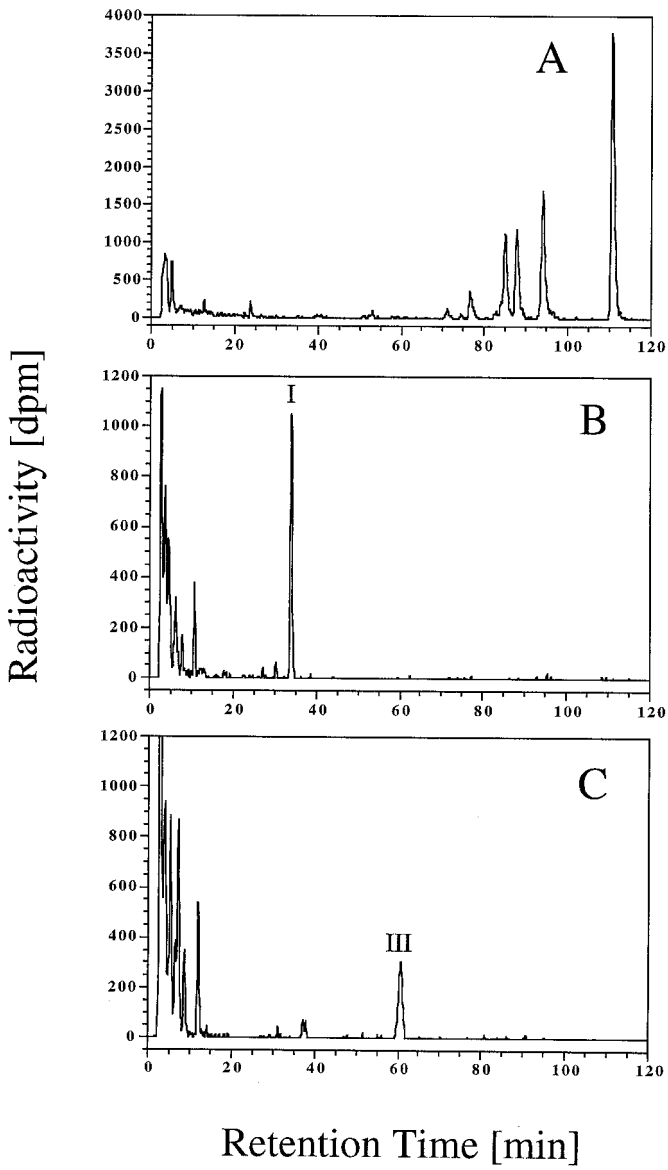

Fig. 3. HPLC elution profiles of ^{33}P-labeled DNA adducts formed in V79 Chinese hamster cells stably expressing human P450 1A1 24 h after treatment with 5 μM (+)-*trans*-11,12-diol (*A*) or 24 h after treatment with 2.5 μM (+)-*trans*-8,9-diol (*B*) or (−)-*trans*-8,9-diol (*C*). *Peaks I* (*B*) and *III* (*C*), polar DNA adducts derived from enantiomeric *trans*-8,9-diols, as in Fig. 2. HPLC conditions are described in "Materials and Methods."

by human P450 1A1 and 1B1 coelute (data not shown). That suggests that these adducts are formed by the same reactive metabolite(s).

DISCUSSION

Shortly after the first successful synthesis of DB[*a,l*]P in 1966 (17, 18), carcinogenicity studies demonstrated that this PAH possesses extraordinarily high carcinogenic potency (19, 20). Twenty years later, several investigations confirmed the high carcinogenic potency of DB[*a,l*]P in mouse skin and rat mammary gland compared with that of other carcinogenic PAH, including benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene (1–4). DB[*a,l*]P, like other PAHs, exerts genotoxic effects through DNA adduct formation in biological systems only after metabolism to reactive intermediates such as vicinal diol epoxides (21–23). The oxidative steps in metabolic activation of carcinogenic PAH are catalyzed by P450 enzymes, especially P450 1A1 and 1B1 (10, 11, 24–28). Whereas P450 1A1 is expressed in substantial amounts in most tissues only after induction by compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, MC, polychlorinated biphenyls (Aroclor 1254), or PAHs (24, 29), P450 1B1 is constitutively expressed in many extrahepatic tissues, including brain, lung, and kidney (28, 30).

Human P450 1A1- (15) or P450 1B1-expressing V79 Chinese hamster cell lines (10) were previously used to elucidate the role of both human enzymes in catalyzing the formation of DB[*a,l*]P-DNA adducts (10). Whereas human P450 1B1 metabolically activated DB[*a,l*]P almost exclusively on the fjord region to produce high amounts of (−)-*anti*-DB[*a,l*]PDE and low amounts of (+)-*syn*-DB[*a,l*]PDE, human P450 1A1 formed adducts of both DB[*a,l*]PDEs as well as several highly polar DNA adducts (10). The same adducts were also formed after exposure of human P450 1A1-expressing cells to the (−)-*trans*-11,12-diol (Fig. 1), the immediate metabolic precursor of the (−)-*anti*-DB[*a,l*]PDE (10). The improved HPLC gradient used for DNA adduct separation in the present studies confirmed these differences in activation by P450 1A1 and 1B1.

Theoretically, DB[*a,l*]P could be enzymatically converted to three different diol epoxides, namely the bay region 1,2-diol 3,4-epoxide, the fjord region 3,4-diol 1,2-epoxide, and the fjord region 11,12-diol 13,14-epoxide (Fig. 1). Moreover, each of these diol epoxides could exist as a *syn*- or *anti*-diastereoisomers, and an additional number of phenolic or higher hydroxylated derivatives could also be formed. However, only the 7-phenol and the *trans*-diols at positions 8–9, 11–12, and 13–14 of the hexacyclic ring system were detected after incubation of DB[*a,l*]P with rat (12) or human (11) P450 1A1-containing microsomes. Direct incubation of enantiomeric *trans*-11,12-diols with P450 1A1-containing microsomes from rats revealed that these substrates were further metabolized to several metabolites in addition to the fjord region 11,12-diol 13,14-epoxides (8). Metabolism at other sites on the DB[*a,l*]P molecule could account for the presence of several highly polar DNA adducts in addition to the DB[*a,l*]PDE-derived adducts after treatment of cells expressing rat P450 1A1 with the (−)-*trans*-11,12-diol (10). Recently, investigations of the metabolism of the K-region *trans*-8,9-diol of DB[*a,l*]P revealed that this substrate was converted by human P450 1A1- and P450 1B1-expressing V79 cells to several *trans*-8,9:*n,m*-bis-diols (where $n = m + 1$) and *trans*-8,9-diol phenols (14). Using commercially available recombinant human P450 1A1 microsomes, Nesnow *et al.* (13) identified and spectroscopically characterized several diastereomeric *trans*-8,9:11,12- and *trans*-8,9:13,14-bis-diols as products of metabolism of the K-region *trans*-8,9-diol. The ability of human P450 1A1 to successively metabolize different benzo rings in the pericondensed hexacyclic DB[*a,l*]P is consistent with previous findings that several other penta- or hexacyclic hydrocarbons, such as benzo[*a*]pyrene (31), benz[*a*]anthracene (32), dibenz[*a,c*]anthracene (33), DB[*a,h*]A (33–35), DB[*a,j*]A (33), and dibenzo[*a,h*]pyrene (36) are metabolized by P450 1A1 on different benzo rings in the same molecule.

Among the many polyhydroxylated products metabolically formed from DB[*a,j*]A and DB[*a,h*]A, only one metabolite containing a diol group at the K-region in addition to further diol groups at other regions was observed (33, 34). DB[*a,j*]A, which possesses two K-regions at positions 5–6 and 8–9, can be metabolically converted to low amounts of the *trans*-3,4:8,9-bis-diol (33). However, only DB[*a,j*]A-DNA adducts derived from the bay region 3,4-diol 1,2-epoxide and from the K-region 5,6-oxide could be detected in cells in culture (37). Recently, a mouse skin study demonstrated that these bay region diol epoxide-DNA adducts are the major adducts formed (38). The highly polar DB[*a,j*]A-DNA adducts formed in lower amounts resulted mainly from further activation of the *trans*-3,4-diol and

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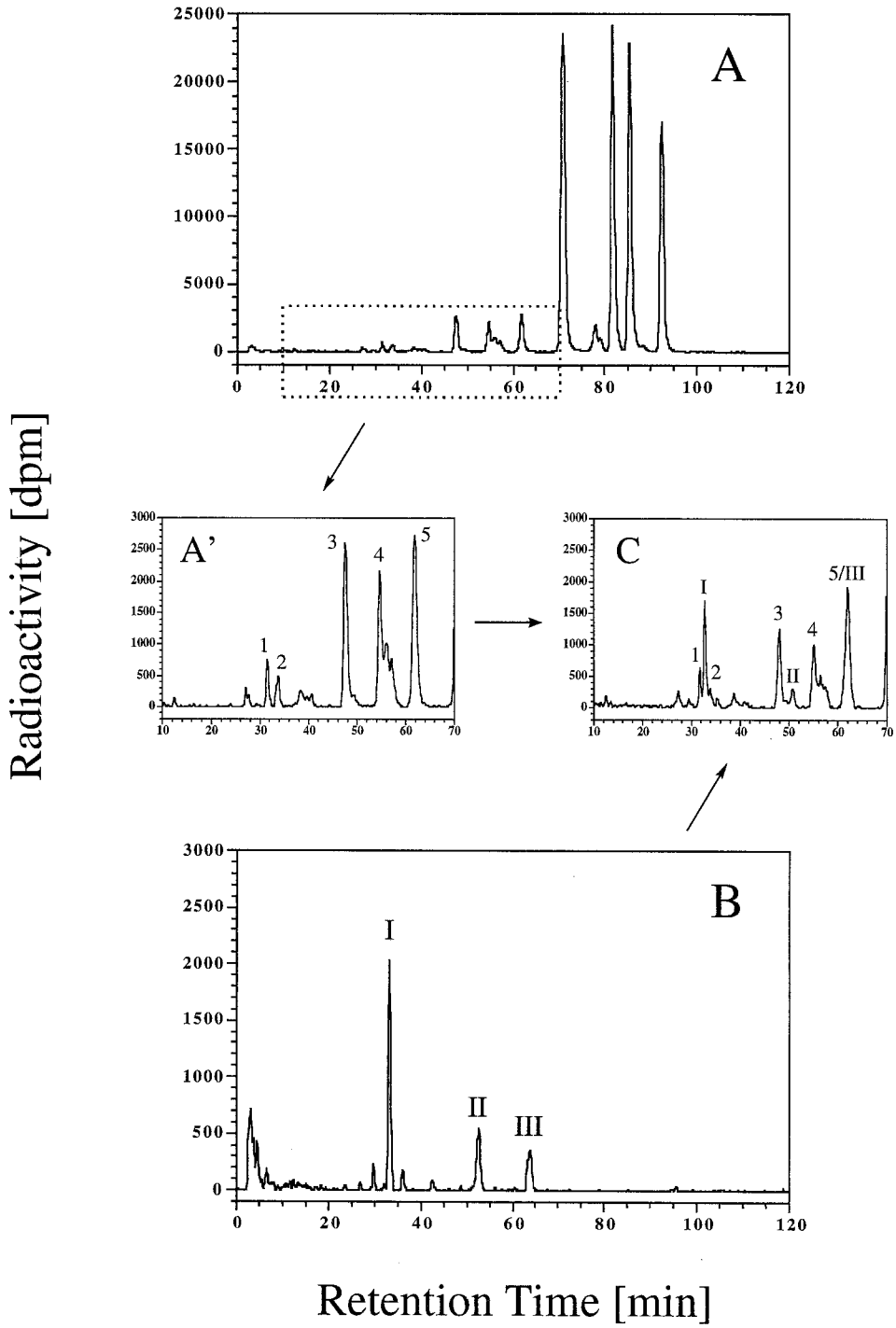

Fig. 4. HPLC elution profiles of ^{33}P-labeled DNA adducts formed in V79 Chinese hamster cells stably expressing human P450 1B1 6 h after treatment with 0.05 μM (−)-*trans*-11,12-diol (*A*) or 24 h after treatment with 5 μM (±)-*trans*-8,9-diol (*B*). *A′*, enlarged area marked in *A*. *Peaks 1–5*, highly polar unidentified (−)-*trans*-11,12-diol-derived DNA adducts; *peaks I–III*, (±)-*trans*-8,9-diol-derived DNA adducts. The HPLC elution profile of a mixture of aliquots of samples *A* and *B* (in a ratio of 1:1) is shown in *C*. HPLC conditions are described in "Materials and Methods."

trans-3,4:10,11-bis-diol, respectively (38). However, a very low level (~0.5% of the total DNA binding in mouse skin) resulted from activation of DB[*a,j*]A through the *trans*-3,4:8,9-bis-diol (38). The strong carcinogen DB[*a,h*]A with two K-regions at positions 5–6 and 12–13 was converted by P450 1A1-containing microsomes to low amounts of the *trans*-3,4:12,13-bis-diol through intermediate formation of the *trans*-3,4-diol (34). In the same study, two other bis-diols, the *trans*-3,4:8,9- and *trans*-3,4:10,11-bis-diols of DB[*a,h*]A, were also detected. However, the highly hydroxylated DB[*a,h*]A-DNA adducts formed *in vitro* (39, 40) or in mouse skin (41) were derived only by successive oxidations of DB[*a,h*]A at positions 3,4 and positions 10,11 to form the *trans*-3,4:10,11-bis-diol. The greater mutagenic activity of the *trans*-3,4:10,11-bis-diol than the *trans*-3,4-diol and the lack of any mutagenic activity of the *trans*-3,4:12,13-bis-diol reported by Platt and Schollmeier (34) were consistent with this activation pathway. The hexacyclic hydrocarbon dibenzo[*a,h*]pyrene was found to be metabolized in mouse skin only at benzo rings *a* and *h*; thus, the K-regions located at positions 5–6 and 12–13 were not involved in metabolic formation of reactive intermediates that bound to DNA (36). These results, together with previous studies on K-region epoxides (42–44), lead to the conclusion that enzymatic oxidation in the K-region is usually a detoxification step rather than an activation step toward formation of DNA-binding intermediates of PAH.

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Human or rat P450 1A1-expressing V79 Chinese hamster cells have been shown to metabolize the strongly carcinogenic hexacyclic DB[*a*,*l*]P to reactive metabolite(s) that formed several highly polar DNA adducts (10). The modified Sep-Pak elution and HPLC gradient used in the present studies provided improved resolution of the polar DNA adducts formed in human P450 1A1- and 1B1-expressing cells. In both cell lines, highly polar DNA adducts were observed after incubation with DB[*a*,*l*]P and (−)-DB[*a*,*l*]P-*trans*-11,12-diol (Figs. 2 and 4). The relative proportion of these adducts compared with DB[*a*,*l*]PDE adducts in human P450 1A1-expressing cells was considerably higher than that in human P450 1B1-expressing cells (Table 1). In both cell lines, the patterns of highly polar DNA adducts derived from the parent PAH and (−)-DB[*a*,*l*]P-*trans*-11,12-diol were identical (Fig. 2, *A* and *B*, and Fig. 4*A*). Comparison of these adducts to the DNA adducts derived from the K-region *trans*-8,9-diol of DB[*a*,*l*]P revealed that the major *trans*-8,9-diol-derived DNA adduct (Figs. 2 and 4, *peak I*, in both cell lines) was not the same as a major polar DNA adduct derived from DB[*a*,*l*]P or its (−)-*trans*-11,12-diol. In human P450 1A1-expressing cells the *trans*-8,9-diol-derived DNA adduct I was not identical to the DB[*a*,*l*]P- or (−)-*trans*-11,12-diol-derived DNA adduct 1 (Fig. 2). Nesnow *et al.* (13) used a recombinant human P450 1A1 microsomal system to study activation of the *trans*-8,9-diol of DB[*a*,*l*]P. They found that the K-region *trans*-8,9-diol was metabolized to *trans*-8,9:11,12- and *trans*-8,9:13,14-bis-diols, suggesting that further metabolic activation to bis-diol epoxides could be involved in DNA binding (13). Analysis of the DNA adducts formed from the *trans*-8,9-diol upon activation in this microsomal system by HPLC indicated that the major *trans*-8,9-diol-DNA adduct coeluted with the DB[*a*,*l*]P-derived highly polar DNA adduct. However, the gradient used provided more limited resolution of these adducts (13) than did that used here. HPLC analyses of the DNA adducts formed in human P450 1A1-expressing cells treated with DB[*a*,*l*]P, (−)-DB[*a*,*l*]P-*trans*-11,12-diol, and DB[*a*,*l*]P-*trans*-8,9-diol (Fig. 2) demonstrate that the *trans*-8,9-diol-derived DNA adduct and the highly polar DB[*a*,*l*]P-derived DNA adduct can be separated (Fig. 2*D*, *peaks 1* and *I*), despite their similar polarities and retention times. The extended HPLC gradient used in this study permitted the discrimination of these DNA adducts. The absence of any highly polar DNA adducts with similar retention times in DNA from human P450 1A1-expressing cells treated with (+)-DB[*a*,*l*]P-*trans*-11,12-diol (Fig. 3*A*) indicates that the major DNA adduct derived from the *trans*-8,9-diol does not result from activation through either *trans*-11,12-diol.

The K-region *trans*-8,9-diol of DB[*a*,*l*]P forms very low levels of DNA adducts when activated by either human P450 1A1 or 1B1 (Table 1). At concentrations of 2.5 μM or greater, this compound can be converted by human P450 1A1 and 1B1 to reactive intermediates that form three different DNA adducts (Figs. 2*C* and 4*B*). Incubation of living cells with the very high concentrations of between 2.5 and 10 μM of the *trans*-8,9-diol was possible due to the lack of any cytotoxicity (14). Shou *et al.* (11) previously reported that human P450 1A1 catalytically converts DB[*a*,*l*]P to its *trans*-11,12-diol with an activity of 116 pmol/min·nmol P450 but to its K-region *trans*-8,9-diol with an activity of only ~12 pmol/min·nmol P450. This suggests that the amount of the *trans*-11,12-diol formed from DB[*a*,*l*]P in human P450 1A1-expressing cells is 10-fold higher than the amount of the K-region *trans*-8,9-diol. Detectable DNA damage in human P450 1A1-expressing cells was only observed during incubation with ≥2.5 μM of the *trans*-8,9-diol (Table 1). Thus, during exposure to DB[*a*,*l*]P, the concentration of the *trans*-11,12-diol enzymatically formed in these cells theoretically should reach a concentration of 25 μM before any detectable DNA damage caused by *trans*-8,9-diol-derived metabolites would be observed. This would not occur in viable cells due to the high toxicity of the *trans*-11,12-diol to human P450 1A1-expressing V79 cells (EC_{50} of ~1.4 nM; Ref. 14).

DB[*a*,*l*]P is a very strong carcinogen in mouse skin and rat mammary gland. Intramammillary injection of this PAH in rats and measurement of the DNA adducts formed in several organs 2 days later revealed that only fjord region DB[*a*,*l*]PDE-derived DNA adducts were detectable in mammary, lung, heart, bladder, and pancreas (45). However, four additional highly polar DNA adducts were found in the liver (45). Formation of only DB[*a*,*l*]PDE-DNA adducts in extrahepatic tissues of rats would be consistent with the constitutive expression or induction of P450 1B1 in these extrahepatic organs (28, 46, 47). On the other hand, the major P450 enzyme involved in PAH metabolism in the liver of rats, P450 1A1 (48, 49), could catalyze the formation of highly polar DB[*a*,*l*]P-DNA adducts in this organ. This finding *in vivo* would be consistent with results obtained in cell culture demonstrating that these polar adducts are formed by both rat and human P450 1A1 (10). After the *trans*-11,12-diol, the 7-phenol is the metabolite formed in the greatest amount after incubation of human P450 1A1-containing microsomes with DB[*a*,*l*]P (11). Both metabolites are also formed in high amounts during incubation of MC-induced rat liver microsomes that contain high amounts of rat P450 1A1 (12). The fact that position 7 in DB[*a*,*l*]P is easily oxidized by P450 1A1, together with the finding that an unidentified *trans*-11,12-diol phenol was detected in human P450 1A1-expressing V79 cells after treatment with DB[*a*,*l*]P (14), suggest that the *trans*-(11*R*,12*R*)-diol 7-phenol of DB[*a*,*l*]P warrants further investigation as a possible metabolic intermediate in the formation of polar DNA adducts of DB[*a*,*l*]P.

This study has demonstrated that the highly polar DNA adducts found in human P450 1A1-expressing cells in high amounts after exposure to the strong carcinogen DB[*a*,*l*]P are generated through formation of the (−)-*trans*-11,12-diol. Formation of the K-region *trans*-8,9-diol is not an initial step in the genotoxicity of DB[*a*,*l*]P through activation of the parent compound to either the *trans*-8,9:11,12- or *trans*-8,9:13,14-bis-diols. The activation pathways of the potent carcinogens DB[*a*,*l*]P and DB[*a*,*h*]A are similar in that bis-diols formed from a diol in the K-region, namely, the *trans*-8,9:11,12-bis-diol of DB[*a*,*l*]P and the *trans*-3,4:12,13-bis-diol of DB[*a*,*h*]A, do not contribute to the DNA binding of the parent compound in human cells.

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