

Formation of Benzylic–DNA Adducts Resulting from 7,12-Dimethylbenz[*a*]anthracene in Vivo

M. N. V. Ravi Kumar,^{†,‡} Manicka V. Vadhanam,[†] Jamie Horn,[§]
James W. Flesher,^{*,§} and Ramesh C. Gupta^{*,†}

Department of Pharmacology and Toxicology, Brown Cancer Center, University of Louisville,
Louisville, Kentucky 40202, and Department of Molecular and Biomedical Pharmacology,
University of Kentucky Medical Center, Lexington, Kentucky 40536

Received November 11, 2004

Studies were undertaken to determine the formation of benzylic–DNA adducts in rats administered 7,12-dimethylbenz[*a*]anthracene (DMBA) and its *meso*-region metabolites by subcutaneous injection. Here, we show that 7-hydroxymethyl-12-methylbenz[*a*]anthracene (7-HMBA) and 7-sulfoxymethyl-12-methylbenz[*a*]anthracene (7-SMBA) gave rise to some benzylic–DNA adducts indistinguishable from adducts formed from DMBA. Adducts were analyzed by butanol enrichment-mediated ³²P-postlabeling assay. Female Sprague–Dawley rats given a combined dose of 420 μmol DMBA/kg b. wt resulted in two major and up to nine minor adducts in the subcutaneous tissue, with chromatographic resemblance to benzylic–DNA adducts prepared in vitro. Subcutaneous administration of 7-HMBA, 7-SMBA, and 7-methyl-12-hydroxymethylbenz[*a*]anthracene (12-HMBA) (210, 42, and 210 μmol/kg b. wt, respectively) each resulted in one major and several minor benzylic–DNA adducts. From cochromatography with reference adducts, it was concluded that the benzylic DNA adduct 4, derived from the parent compound, comigrates with the major adduct from 7-HMBA and 7-SMBA, whereas adducts 2 and 3 comigrate with adducts resulting from 12-HMBA and 7-methyl-12-sulfoxymethylbenz[*a*]anthracene, respectively. These data suggest that 7-sulfoxymethyl- and 12-sulfoxymethyl derivatives produce distinct adducts. Several major and minor diol epoxide-related DNA adducts were also detected. The diol epoxide– and benzylic–DNA adducts were found in a 2:1 ratio. The oral, intraperitoneal, and intramammary treatments with DMBA showed no detectable benzylic adducts in the liver and mammary glands 24 h after the last treatment, although the adduct formation was clearly evident with SMBA and/or HMBA treatments, suggesting that hydroxylation of DMBA to form HMBA may be the rate-limiting step for the *meso*-methyl substitution pathway. The present study clearly demonstrates the in vivo formation of benzylic–DNA adducts from DMBA. The data also reveal the involvement of the 12-methyl group of DMBA in adduct formation.

1. Introduction

DNA¹ adduct formation is generally accepted as a critical step in the mechanism by which polycyclic aromatic hydrocarbons cause mutations resulting in induction of cancer in the target organs (1). Most of the carcinogenic hydrocarbons, including 7,12-dimethylbenz[*a*]anthracene (DMBA),² are metabolized, as an essential first step, by cytochrome P450 (2, 3). However, previous studies have shown that in the second step hydroxymethyl metabolites

of polycyclic aromatic hydrocarbons (PAHs) are transformed to DNA binding *meso*-region benzylic electrophilic sulfate ester metabolites that result in carcinogenesis (4–8). Three additional mechanisms have also been proposed as follows: (i) one-electron oxidation by cytochrome P450 and peroxidases to form PAH radical cations that react with DNA to yield both stable and depurinating adducts (9–11); (ii) formation of bay-region epoxy-dihydrodiol metabolites that interact with DNA resulting in tumorigenesis (12, 13); and (iii) oxidation of *trans*-dihydrodiol metabolites by aldo-ketoreductases to catechols that redox cycle with DNA damaging *o*-quinones, to generate reactive oxygen species (ROS) that also attack DNA (14). The *meso*-region theory proposes that a necessary first step in carcinogenesis by most *meso*-methyl-substituted PAHs is biotransformation to a *meso*-hydroxymethyl metabolite, whereas most hydrocarbons lacking a *meso*-methyl substituent undergo a bioalkylation substitution reaction in the *meso*-anthracenic position(s) or L-region (15). Flesher et al. (7) have previously shown that 7-sulfoxymethyl-12-methylbenz[*a*]anthracene (7-SMBA) is an exceptionally reactive electrophilic mutagen and ultimate carcinogen.

* To whom correspondence should be addressed. (R.C.G.) Tel: 502-852-3682. Fax: 502-852-3662. E-mail: rcgupta@louisville.edu. (J.W.F.) E-mail: jwflesh@uky.edu.

[†] University of Louisville.

[‡] Present address: Department of Pharmaceutics, National Institute of Pharmaceutical Education & Research, SAS Nagar, Mohali-160 062, India.

[§] University of Kentucky Medical Center.

¹ A preliminary report of this work was presented at the annual meeting of the American Association for Cancer Research, Abstract 707, Vol. 43, 2002.

² Abbreviations: DMBA, 7,12-dimethylbenz[*a*]anthracene; 7-HMBA, 7-hydroxymethyl-12-methylbenz[*a*]anthracene; 7-SMBA, 7-sulfoxymethyl-12-methylbenz[*a*]anthracene; 12-HMBA, 7-methyl-12-hydroxymethylbenz[*a*]anthracene; 12-SMBA, 7-methyl-12-sulfoxymethylbenz[*a*]anthracene; 7,12-DHMBA, 7,12-dihydroxymethylbenz[*a*]anthracene; PAH, polycyclic aromatic hydrocarbons; ROS, reactive oxygen species; BP, benzo[*a*]pyrene.

Prior studies from this laboratory demonstrated the formation of benzylic-DNA adducts in subcutaneous (sc) tissue of Sprague-Dawley rats treated with benzo[*a*]pyrene (BP) and its 6-methyl, 6-hydroxymethyl, and 6-acetoxymethyl derivatives (16). Similarly, the electrophilic mutagen and ultimate carcinogen 7-SMBA showed high DNA adduction in vitro, which suggests that SMBA plays a role in the metabolic activation, DNA adduction, and complete carcinogenicity of DMBA (17). Previous studies from this and other laboratories have focused on the 7-hydroxymethyl metabolite of DMBA. In the present paper, we have investigated the possibility that the 12-hydroxymethyl metabolite may also be involved in DNA adduction.

Using a highly sensitive ^{32}P -postlabeling assay, we have now investigated (i) whether DMBA benzylic-DNA adducts can be detected in a target tissue in vivo; (ii) whether to rule out or in the possibilities of 12 derivatives involved in DNA adduction; and (iii) whether DNA adduct patterns produced in vivo following treatments with DMBA and known or postulated intermediates of its benzylic ester pathway are qualitatively identical. The data presented clearly show the involvement of both the 7- and the 12-hydroxymethyl metabolites of DMBA in DNA adduction.

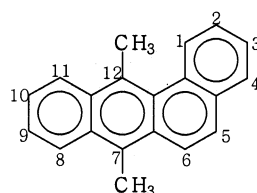
2. Materials and Methods

2.1. Chemicals. DMBA and 7-methyl-12-hydroxymethylbenzo[*a*]anthracene (12-HMBA) were purchased from Sigma Chemical Co. (St. Louis, MO) and the National Cancer Institute (Midwest Research Institute), respectively. Other DMBA metabolites viz. 7-hydroxymethyl-12-methylbenzo[*a*]anthracene (7-HMBA) and 7-SMBA were synthesized in this laboratory as described (7, 17). The materials used in ^{32}P -postlabeling were as described previously (18).

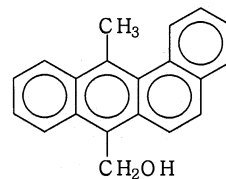
2.2. DNA Adduction in Vitro. Calf thymus DNA was modified in vitro with 7-SMBA as described previously (17). DNA adduction with 7-methyl-12-sulfoxymethylbenzo[*a*]anthracene (12-SMBA) followed the procedure of Vadhanam et al. (17), with modifications. Briefly, to a solution of 12-HMBA (18.4 μmol) in ice-cold dimethylformamide (DMF) was added a 5-fold molar excess of dicyclohexylcarbodiimide (92 μmol) in ice-cold DMF. A 1.5-fold molar excess of sulfuric acid (27.6 μmol) in ice-cold DMF was then added slowly. Aliquots of the reaction mixture were removed at the various time points (30 min and 1, 3, and 6 h) and incubated with calf thymus DNA at 37 °C for 1 h in the presence of 50 mM Tris.HCl, pH 8.0. DNA was recovered by ethanol precipitation and was considered as 12-SMBA-adducted DNA. As a positive control, 12-HMBA was substituted with 7-HMBA. The structures of the various metabolites are shown in Figure 1.

2.3. DNA Adduction in Vivo. Female Sprague-Dawley rats, 28 days old, were obtained from Harlan-Sprague-Dawley, Inc. (Indianapolis, IN). DMBA, 7-HMBA, 7-SMBA, and 12-HMBA (total dose of 420, 210, 42, and 210 $\mu\text{mol}/\text{kg}$, body wt, respectively) were administered sc in 100 μL of DMSO on four alternate days. A group of animals was treated with BP (420 $\mu\text{mol}/\text{kg}$, body wt) to serve as a positive control. Control animals received vehicle only. One day after the last injection, animals were euthanatized by CO_2 asphyxiation/cervical dislocation. The dorsal skin area bound by the shoulder blades and the hind-quarters was removed, and the subcutaneous tissue, which appears as a thin connective tissue lying underneath the dermis, was excised. The tissue was snap frozen in liquid nitrogen and stored at -80 °C until DNA isolation.

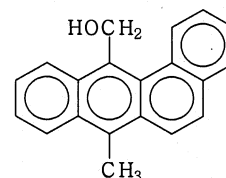
DNA was isolated by a rapid solvent extraction procedure. Briefly, the frozen tissue was suspended in 50 mM Tris.HCl-1mM EDTA (pH 8.0), minced thoroughly with scissors, and then homogenized with Polytron tissue grinder. The homogenate was



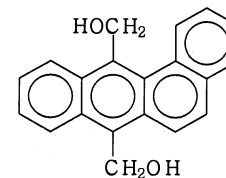
7,12-Dimethylbenzo[*a*]anthracene (DMBA)



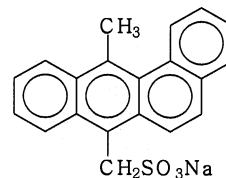
7-Hydroxymethyl-12-methylbenzo[*a*]anthracene (7-HMBA)



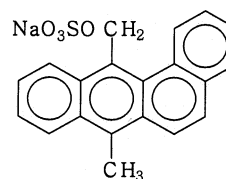
7-Methyl-12-hydroxymethylbenzo[*a*]anthracene (12-HMBA)



7,12-Dihydroxymethylbenzo[*a*]anthracene (7,12-DHMBA)



7-Sulfoxymethyl-12-methylbenzo[*a*]anthracene (7-SMBA)



7-Methyl-12-sulfoxymethylbenzo[*a*]anthracene (12-SMBA)

Figure 1. Structures of DMBA and its various metabolites.

centrifuged at 1000g to obtain a crude nuclear pellet, which was suspended in 50 mM Tris.HCl-1 mM EDTA (pH 8.0). Subsequent treatment with RNases A/T1 and proteinase K removed residual RNA and protein. The mixture was then extracted sequentially with equal volumes of phenol, phenol/Sevag (1:1), and Sevag. The DNA was precipitated by adding sodium chloride and ethanol and dissolved in HPLC grade water. The DNA concentration was measured spectrophotometrically on the basis that one A_{260} equals 50 μg of DNA.

2.4. Adduct Enrichment. The commonly used nuclease P1 and butanol extraction adduct enrichment procedures were first compared using 7-SMBA DNA adduct to determine the maximal adduct recoveries. Both procedures provided qualitatively similar adduct patterns; however, the extraction procedure gave consistently higher recoveries. Therefore, in all subsequent analyses, adducts were enriched with butanol extraction.

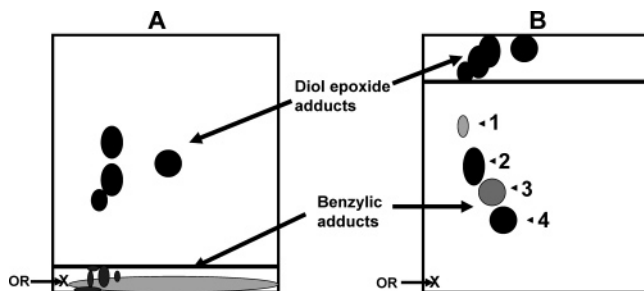


Figure 2. Schematic representation for separating diol epoxide-related and benzylic DNA adducts resulting from DMBA by PEI-cellulose TLC. Solvents were as follows: D1 = 0.5 M sodium phosphate, pH 6.0 (A, B); D2 = 4 M lithium formate-7 M urea, pH 3.5 (A), or 0.7 M ammonium hydroxide (B); D3 = *iso*-propanol:4 M ammonium hydroxide, 1.4:1 (A), or *iso*-propanol:4 M ammonium hydroxide, 1.2:1 (B); and D5 = 1 M sodium phosphate, pH 6.0 (A, B). OR denotes origin.

2.5. Analysis of Adducts. DNA adducts were analyzed by the ^{32}P -postlabeling assay after enrichment of adducts by butanol extraction (19). Briefly, DNA was hydrolyzed to deoxyribonucleoside 3'-monophosphates by incubation with a mixture of micrococcal nuclease and spleen phosphodiesterase (enzyme: substrate, 1:5; 37 °C; 5 h). Adducts were extracted with butanol and ^{32}P -labeled by [γ - ^{32}P]ATP (~1.2 μM ; ~150 μCi ; ~6000 Ci/mmol) and T4 polynucleotide kinase (0.2 U/ μL) phosphorylation. Labeled adducts were resolved by multidirectional PEI-cellulose TLC (20, 21), with modifications (17), using solvents described in the Figure 1 legend. Normal nucleotides were also labeled in parallel with adducts and resolved by one directional PEI-cellulose TLC. Adducts were visualized and quantified by Packard InstantImager (Packard Instrument Co., Downers Grove, IL). The relative adduct labeling was calculated as: $\text{RAL} = [\text{cpm of adduct}/\text{cpm of total nucleotides}] \times 1/\text{dilution factor}$. The adduct levels are expressed as adduct(s)/ 10^9 nucleotides.

3. Results

3.1. TLC Separation Scheme. An integral part of the ^{32}P -postlabeling DNA adduct assay is the multidirectional anion exchange PEI-cellulose TLC, which provides excellent separation of lipophilic adduct nucleotides from the relatively polar normal nucleotide contaminants (18, 20). This chromatography scheme, however, required substantial alterations in order to separate the highly lipophilic benzylic-DNA adducts from the more polar diol epoxide-related adducts. After numerous trials, we developed a solvent system (Figure 2) to achieve a clear separation of the diol epoxide-DNA adducts from the highly lipophilic benzylic-DNA adducts. The chromatography involves a three-step development, in which the chromatogram is developed in a standard high-salt solution (D1) to remove unadducted nucleotides and other contaminants while adducts that are lipophilic remain at the origin. The diol epoxide-DNA adducts are then selectively eluted in high-salt, high-urea solvents (D2), followed by elution of the more lipophilic benzylic-DNA adducts in dilute ammonium hydroxide (D3), and then partitioned in 2-propanol-dilute ammonium hydroxide (D4). An additional development (D5) in high-salt solvent allows the removal of residual background radioactivity.

3.2. DNA Adducts Formed from DMBA Metabolites in Vitro. The adduct pattern obtained by incubation of DNA with 7-SMBA was chromatographically identical to the adduct profile obtained from the reaction mixture of 7-HMBA and sulfuric acid with DNA. In both cases, one major and several minor adducts were detected and the adduct patterns were essentially similar to our

published data (17). These data suggest that the novel reaction scheme to trap the esterified metabolite, 7-SMBA, can be adopted to trap DNA reactive metabolite(s) that may otherwise be unstable for isolation. One major and at least 10 minor adducts were also detected when the reaction mixture of 12-HMBA and sulfuric acid was incubated with DNA; the major adduct accounted for >70% of the total adduction. Of the 30 min and 1, 3, and 6 h reaction kinetics monitored, the maximal DNA adduction occurred after 1 and 3 h for the 12- and 7-HMBA metabolites, respectively. The in vitro adduct patterns for both 7- and 12-SMBA are shown along with the in vivo data below (Figure 3).

3.3. Formation of Benzylic-DNA Adducts in Subcutaneous Tissue following sc Treatment with DMBA and Its Metabolites. Employing the optimized ^{32}P -postlabeling conditions, adducts were quantified using 5 μg of DNA; the amount of DNA was reduced to 0.05 μg for the esterified metabolites because of the much higher levels of benzylic-DNA adducts expected. Two major (#2 and #4) and up to nine minor adducts were detected from DMBA under the chromatographic conditions selected for benzylic adducts (Figure 3A); under these conditions, the more polar, diol epoxide-related adducts migrated toward the top of the chromatogram (not shown). 7-HMBA and 12-HMBA both resulted in one major and several minor adducts; however, the major adducts were clearly chromatographically distinct (Figure 3B,C). The adduct pattern from 7-SMBA was chromatographically similar to the adduct pattern observed with 7-HMBA (Figure 3D). Cochromatography analyses showed that the major adduct (#4) of the parent compound cochromatographed with the major adduct of both 7-HMBA and 7-SMBA (Figure 3G and I). Another major adduct (#2) of the parent compound cochromatographed with the major adduct of 12-HMBA (Figure 3H), and a minor adduct (#3) cochromatographed with the major adduct of 12-SMBA (Figure 3J). Essentially similar conclusions were drawn when the solvent in direction D3 was substituted with 0.6 M sodium bicarbonate and 7 M urea, pH 10.0 (data not shown). These results suggest that the two major and one minor adducts of DMBA (#2-4) are derived from further activation of 7- and 12-HMBA. The other minor adducts of DMBA (#1 and #5-11) did not clearly separate from the minor adducts of either 7-HMBA or 12-HMBA.

When the in vivo adducts were resolved under conditions selected for adducts derived from the diol epoxide pathway, seven major and minor adducts were detected (not shown) consistent with our published data (22). Under these chromatographic conditions, no adducts were detected from 7- and 12-HMBA. Measurement of the diol epoxide and benzylic-DNA adducts revealed that the DMBA-derived benzylic-DNA adducts accounted for nearly one-half of the diol epoxide-related adducts (Figure 4). Treatment with B[a]P, included as a positive control, resulted in essentially the same adduct pattern of benzylic-DNA adducts as published (16).

3.4. Formation of Benzylic-DNA Adducts following Treatment with DMBA and Some of Its Metabolites following Other Routes of Administration. No detectable benzylic-DNA adducts were found either in the mammary glands or in the liver DNA 24 h following treatment with DMBA (420 $\mu\text{g}/\text{kg}$ body wt) administered intraperitoneally or by intramammary route, although the adduct formation was clearly evident with SMBA

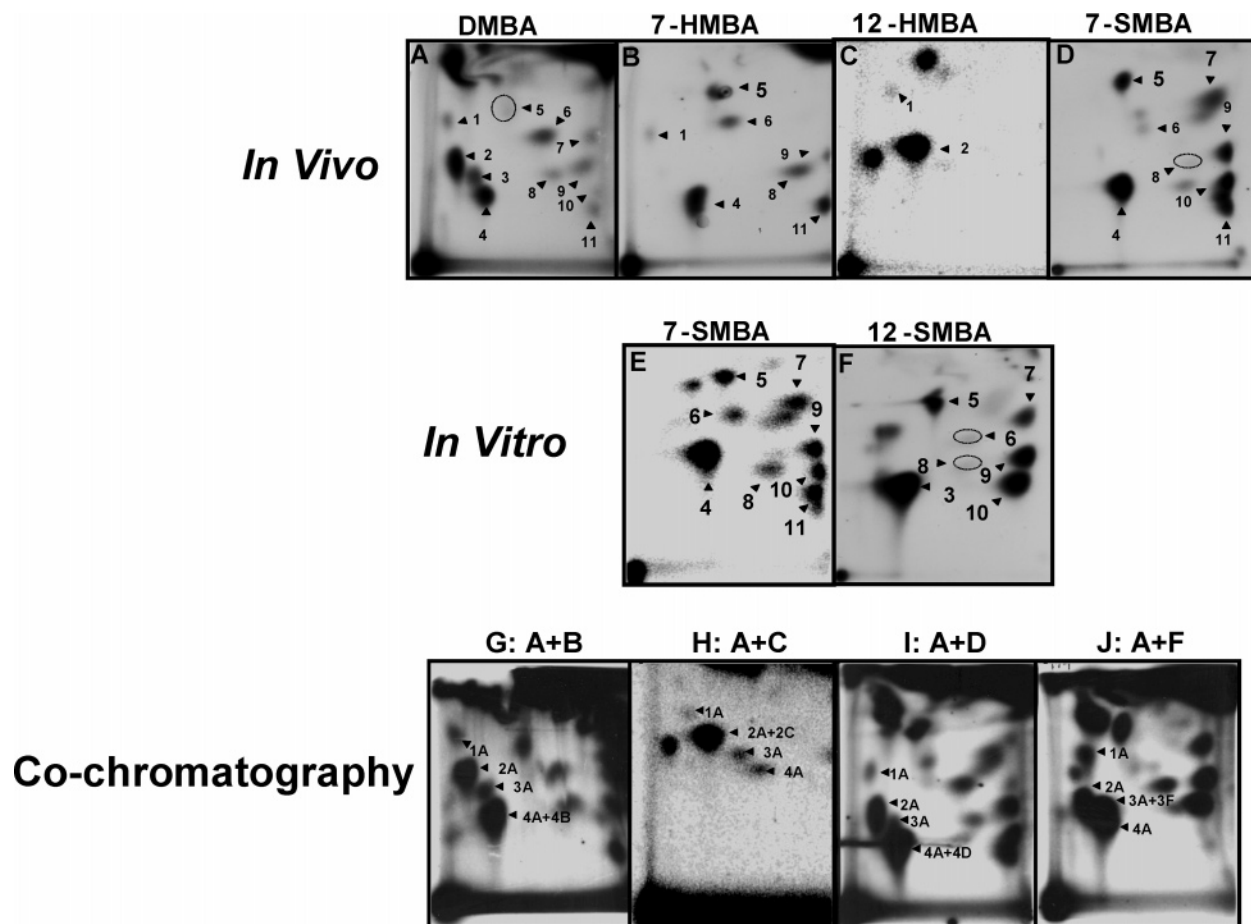


Figure 3. Representative maps of ^{32}P -labeled benzylic adducts in subcutaneous tissue DNA of rats treated with DMBA and its metabolites. The adducts were resolved by 2D PEI-cellulose TLC using solvents described in Figure 1B.

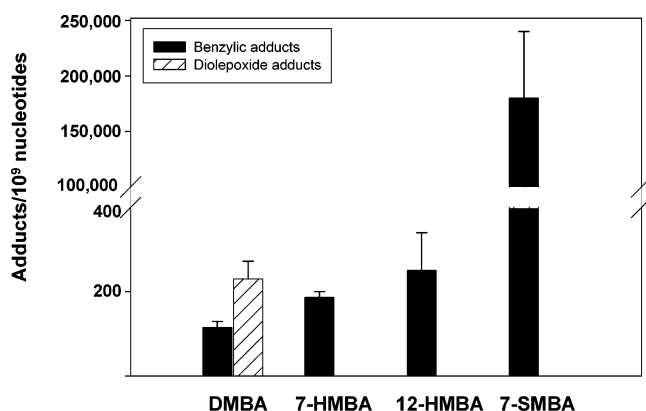


Figure 4. Levels of diol epoxide and benzylic adducts formed in vivo after subcutaneous treatment with DMBA and its metabolites. Values are means of four replicates, and the levels are expressed as adducts/ 10^9 nucleotides.

and/or HMBA (not shown). Similar results were obtained when DMBA was administered orally (65 mg/kg body wt). However, in agreement with previous studies (22), the diol epoxide-related adducts were readily detected in both of these tissues irrespective of the route of treatment (not shown). Our data suggest that in these tissues, hydroxylation of DMBA to form HMBA is probably the rate-limiting step for benzylic-DNA adducts formation.

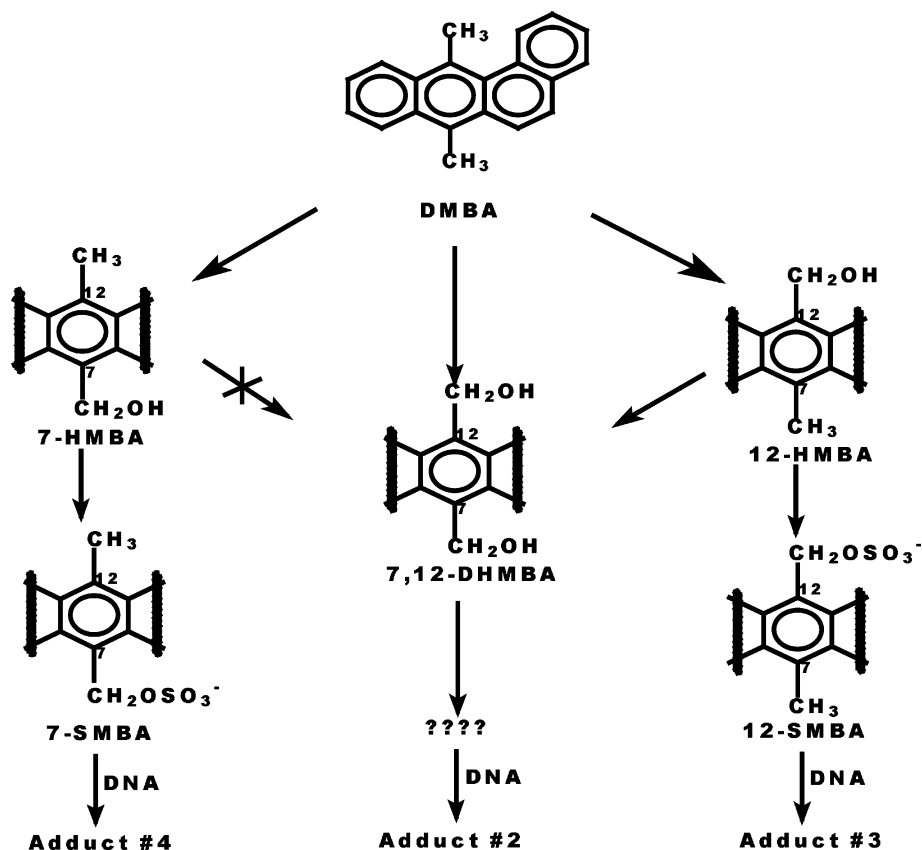
4. Discussion

It is generally accepted that the formation of DNA adducts by covalent binding of electrophilic species of

carcinogens with macromolecules, particularly DNA, is an essential step in the process of carcinogenesis (23). We have demonstrated that 12-SMBA forms DNA adducts, which can be considered as one of the most significant findings of the current investigations, the primary being the demonstration of in vivo evidence for the formation of benzylic-DNA adducts. Our in vivo data showed that adducts #2-4 are formed by either of the 7- or 12-hydroxymethyl metabolites of DMBA but most other minor adducts #1 and #5-11 are common to both of these metabolites as well as DMBA, presumably due to the lack of separation under the present chromatographic conditions. From the data, it is clear that a complex mechanism is involved in the DMBA metabolism. On the basis of our in vivo benzylic adducts obtained from DMBA, it appears that it could be metabolized in three different ways (Scheme 1). Adduct 2 from the parent compound migrates with adduct 2 of 12-HMBA, but not with that of 12-SMBA, suggesting a possibility of 7,12-dihydroxymethylbenz[*a*]anthracene (7,12-DHMBA) being involved in DNA adduction, whereas adducts 3 and 4 are related to 12-SMBA and 7-SMBA, respectively, as proposed in the Scheme 1. The postulated 7,12-DHMBA metabolite could undergo estrification at either the 7- or the 12-hydroxymethyl group to form adduct #2. However, our attempts to prepare these metabolites and their corresponding benzylic-DNA adducts were not successful.

One-electron oxidation of DMBA gives rise to benzylic alcohol and *meso*-aldehyde metabolites (24). The results are consistent with the hypothesis that hydroxylation of

Scheme 1. Plausible Mechanism for DMBA-Mediated DNA Adduction in Subcutaneous Tissue



the 7-methyl and/or 12-methyl groups is the first metabolic step in DMBA activation. (24). Thus, the 12-hydroxymethyl derivative can be formed and can be esterified and be a DNA-binding metabolite. The observations support the hypothesis that benzylic-DNA adduct formation plays a significant role in the carcinogenesis process (16). The complete carcinogenicity data show that 7-SMBA, a metabolite of 7-HMBA, is an exceptionally reactive electrophilic mutagen and ultimate carcinogen (7). The benzylic derivatives of all the metabolites tested were highly electrophilic and formed high adduction with both DNA and deoxynucleoside 3'-phosphates (17).

It has been shown that the formation of 7-HMBA from DMBA and its subsequent esterification generate reactive electrophile (4). SMBA is an electrophilic mutagen in bacterial mutagenicity assays and generates DNA adducts when given to rats and mice (25). It has also been shown that in both rats and rat liver cytosol, hydroxysteroid sulfotransferase activity is probably responsible for sulfonation and bioactivation of 7-HMBA to a reactive electrophile and that at least six different forms of the enzyme exists (25). In contrast to these reports, Surh et al (26) could not find convincing evidence that the electrophilic mutagen SMBA plays a role in carcinogenesis by either 7-HMBA or DMBA. On the other hand, this group has shown that sulfoxymethylbenz[a]pyrene plays a major role in the metabolic activation and carcinogenicity of 6-hydroxymethylB[a]P. Furthermore, we have identified SMBA as an ultimate electrophile and carcinogenic form of 7-HMBA. Flesher et al. (7), supporting their hypothesis, have demonstrated that within 52 weeks, a total dose of 4 μmol of 7-SMBA induced sarcomas at the site of injection in 12 out of 12 rats with

an average induction time of 29 weeks; however, a total dose of 4 μmol of 7-HMBA induced sarcomas in only nine out of 12 rats with an average induction time of 30 weeks.

This is the first report demonstrating the presence of benzylic DNA adducts in vivo. Their presence in the subcutaneous tissues suggests that these adducts may be biologically relevant to tumorigenesis. However, their absence in other target tissues (mammary) suggests that this bioalkylation pathway is tissue specific.

Our data strongly suggest the existence of both the diol epoxide and the benzylic ester pathways in the biotransformation of DMBA in the subcutaneous tissue. Previous studies from this and other laboratories focused only on 7-hydroxy derivatives of DMBA, but our data clearly indicate the involvement of 12-hydroxy derivatives in DNA adduction as well, which opened a new avenue in the DMBA metabolism and its carcinogenicity.

Acknowledgment. This work was supported by NIH/NCI Grant CA85134 and in part by the Agnes Brown Duggan Endowment Funds.

References

- (1) Dipple, A., Moschel, R. C., and Bigger, C. A. H. (1984) Polynuclear aromatic carcinogens. In *Chemical Carcinogens*, 2nd ed. (Searle, C. E., Ed.) ACS Monographs 182, pp 41-174, American Chemical Society, Washington, DC.
- (2) Kadlubar, F. F., and Hammons, G. J. (1987) The role of cytochrome P-450 in the metabolism of chemical carcinogens. In *Mammalian Cytochromes P-450* (Guengerich, F. P., Ed.) pp 81-130, CRC Press, Boca Raton, FL.
- (3) Christou, M., Moore, C. J., Gould, M. N., and Jefcoate, C. R. (1987) Induction of mammary cytochromes P-450: An essential first step in the metabolism of 7,12-dimethylbenz[a]anthracene by rat mammary epithelial cells. *Carcinogenesis* 8, 73-80.

- (4) Flesher, J. W., and Sydnor, K. L. (1971) Carcinogenicity of derivatives of 7,12-dimethylbenz[a]anthracene. *Cancer Res.* 31, 1951–1954
- (5) Flesher, J. W., and Sydnor, K. L. (1973) Possible role of 6-hydroxymethylbenzo[a]pyrene as a proximate carcinogen of benzo[a]pyrene and 6-methylbenzo[a]pyrene. *Int. J. Cancer* 11, 433–437.
- (6) Surh, Y. J., Liem, A., Miller, E. C., and Miller, J. A. (1989) Metabolic activation of the carcinogen 6-hydroxymethylbenzo[a]pyrene: Formation of an electrophilic sulfuric acid ester and benzylic DNA adducts in rat liver in vivo and in reactions in vitro. *Carcinogenesis* 10, 1519–1528.
- (7) Flesher, J. W., Horn, J., and Lehner, A. F. (1997) 7-Sulfooxymethyl-12-methylbenz[a]anthracene is an exceptionally reactive electrophilic mutagen and ultimate carcinogen. *Biochem. Biophys. Res. Commun.* 231, 144–148.
- (8) Flesher, J. W., Horn, J., and Lehner, A. F. (1997) 6-Sulfooxymethylbenzo[a]pyrene is an ultimate electrophilic and carcinogenic form of the intermediary metabolite 6-hydroxymethylbenzo[a]pyrene. *Biochem. Biophys. Res. Commun.* 234, 554–558.
- (9) Cavalieri, E. L., and Rogan, E. G. (1995) Central role of radical cations in metabolic activation of polycyclic aromatic hydrocarbons. *Xenobiotica* 25, 677–688.
- (10) Casale, G. P., Singhal, M., Bhattacharya, S., RamaNathan, R., Roberts, K. P., Barbacci, D. C., Zhao, J., Jankowiak, R., Gross, M. L., Cavalieri, E. L., Small, G. J., Rennard, S. I., Mumford, J. L., and Shen, M. (2001) Detection and quantification of depurinated benzo[a]pyrene-adducts DNA bases in the urine of cigarette smokers and women exposed to household coal smoke. *Chem. Res. Toxicol.* 14, 192–201.
- (11) RamaKrishna, N. V., Devanesan, P. D., Rogan, E. G., Cavalieri, E. L., Jeong, H., Jankowiak, R., and Small, G. J. (1992) Mechanism of metabolic activation of the potent carcinogen 7,12-dimethylbenz[a]pyrene. *Chem. Res. Toxicol.* 5, 220–226.
- (12) Jerina, D. M., Yagi, H., Lehr, R. E., Thakker, D. R., Schaefer-Ridder, M., Karle, J. M., Levin, W., Wood, A. W., Chang, R. L., and Conney, A. H. (1978) The bay-region theory of carcinogenesis by polycyclic aromatic hydrocarbons. In *Polycyclic Hydrocarbons and Cancer: Environment, Chemistry and Metabolism* (Gelboin, H. V., and Ts' O, P. O. P., Eds.) pp 173–188, Academic Press, NY.
- (13) Conney, A. (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. *Cancer Res.* 42, 4875–4917.
- (14) Penning, T. M., Palackal, N. T., Blair, I. A., and Harvey, R. G. (2002) The aldo-keto reductases and polycyclic aromatic hydrocarbons activation. *Polycyclic Aromat. Compd.* 22, 791–800.
- (15) Flesher, J. W., Myers, S. R., and Blake, J. W. (1984) Biosynthesis of the potent carcinogen 7,12-dimethylbenz[a]anthracene. *Cancer Lett.* 24, 335–343.
- (16) Stansbury, K. H., Flesher, J. W., and Gupta, R. C. (1994) Mechanism of aralkyl-DNA adduct formation from benzo[a]pyrene in vivo. *Chem. Res. Toxicol.* 7, 254–259.
- (17) Vadhanam, M. V., Horn, J., Flesher, J. W., and Gupta, R. C. (2003) Detection of benzylic adducts in DNA and nucleotides from 7-sulfooxymethyl-12-methylbenz[a]anthracene and related compounds by ³²P-postlabeling using new TLC systems. *Chem.-Biol. Interact.* 146, 81–87.
- (18) Gupta, R. C. (1993) ³²P-Postlabeling analysis of bulky aromatic adducts. In *Postlabeling Methods for Detection of DNA Adducts* (Phillips, D. H., Castegnaro, M., and Bartsch, H., Eds.), pp 11–23, IARC, Lyon, France.
- (19) Gupta, R. C. (1985) Enhanced sensitivity of ³²P-postlabeling analysis of aromatic carcinogen: DNA adducts. *Cancer Res.* 45, 5656–5662.
- (20) Gupta, R. C., Reddy, M. V., and Randerath, K. (1982) ³²P-Postlabeling analysis of nonradioactive aromatic carcinogen-DNA adducts. *Carcinogenesis* 3, 1081–1092.
- (21) Gupta, R. C. (1996) ³²P-Postlabeling for detection of DNA adducts. In *Technologies in Detection of DNA Damage and Mutations* (Pfeifer, G. P., Ed.) pp 45–61, Plenum Press, NY.
- (22) Devanaboyina, U., Gairola, C. G., Kelloff, G. J., Lubet, R. L., and Gupta, R. C. (1997) Effects of indole-3-carbinol (I3C) and phenethyl isothiocyanate (PEITC) on 7,12-dimethylbenz[a]anthracene (DMBA)-induced DNA adducts in rat mammary glands and liver. *Proc. Am. Assoc. Cancer Res.* 38, 362–363.
- (23) Miller, E. C., and Miller, J. A. (1981) Mechanisms of chemical carcinogenesis. *Cancer* 47, 1055–1064.
- (24) Flesher, J. W., Horn, J., and Lehner, A. F. (2004) Formation of benzylic alcohols and meso-aldehydes by one electron oxidation of DMBA, a model for the first metabolic step in methylated carcinogenic hydrocarbon activation. *Polycyclic Aromat. Compd.* 24, 501–511.
- (25) Watabe, T., Ishizuka, T., Isobe, M., and Ozawa, N. (1982) A 7-hydroxymethyl sulfate ester as an active metabolite of 7,12-dimethylbenz[a]anthracene. *Science* 215, 403–405.
- (26) Surh, Y.-J., Liem, A., Miller, E. C., and Miller, J. A. (1991) 7-Sulfooxymethyl-12-methylbenz[a]anthracene is an electrophilic mutagen, but does not appear to play a role in carcinogenesis by 7,12-dimethylbenz[a]anthracene or 7-hydroxymethyl-12-methylbenz[a]anthracene. *Carcinogenesis* 12, 339–347.

TX049686P