Correlation between Formation of a Specific Hydrocarbon-Deoxyribonucleoside Adduct and Tumor-initiating Activity of 7,12-Dimethylbenz(a)anthracene and Its 9- and 10-Monofluoroderivatives in Mice

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ABSTRACT

The formation of epidermal DNA adducts from 9-fluoro-7,12-dimethylbenz(a)anthracene (9-F-DMBA) was compared with 7,12-dimethylbenz(a)anthracene (DMBA) and 10-fluoro-7,12-dimethylbenz(a)anthracene (10-F-DMBA) in SENCAR mice. 9-F-DMBA is equipotent, whereas 10-F-DMBA is more potent than DMBA for skin tumor initiation in this mouse stock. The quantity of covalently bound DNA adducts was essentially identical between 9-F-DMBA and DMBA at all doses tested in the range of 10 to 100 nmol/mouse. These results correlated closely with the dose-response relationships for tumor initiation by the two hydrocarbons. A quantitative comparison of the hydrocarbon-DNA adducts formed after topical application of 100 nmol of DMBA, 9-F-DMBA, and 10-F-DMBA yielded interesting results. The total binding for the three hydrocarbons at this dose was 16.2 ± 2.6, 18.4 ± 2.4, and 52.3 ± 6.8 pmol/mg of epidermal DNA, respectively. Analysis of these DNA adduct samples by dihydroboronate chromatography demonstrated marked reductions in the percentage of syn-diol-epoxide-DNA adducts with both 9-F-DMBA (24%) and 10-F-DMBA (18%) compared with DMBA (57%).

Analysis of DNA adduct samples from DMBA-, 9-F-DMBA-, and 10-F-DMBA-treated mice (100 nmol/mouse) by high-pressure liquid chromatography revealed qualitatively similar profiles. However, a quantitative comparison of the three major DNA adducts, tentatively identified as anti-diol-epoxide-deoxyguanosine (Peak I), syn-diol-epoxide-deoxyadenosine (Peak II), and anti-diol-epoxide-deoxyadenosine (Peak III), revealed significant differences. With both 9-F-DMBA and 10-F-DMBA there were marked increases (236% and 644%, respectively) in the quantity of Peak I compared to DMBA. On the other hand, Peak II was formed in approximately equal amounts with DMBA and 10-F-DMBA but only 50% of the DMBA value with 9-F-DMBA. Interestingly, Peak III was formed in approximately equal amounts with both DMBA and 9-F-DMBA but was increased to 337% of the DMBA value with 10-F-DMBA. Thus, the actual level of Peak III (tentatively identified as anti-diol-epoxide-deoxyadenosine) correlated closely with the tumor-initiating activity of these three hydrocarbons, whereas the levels of the other two adducts did not. These data suggest that formation of a specific DNA adduct may be important for DMBA skin tumor initiation. These data are discussed in relation to skin tumor initiation by other hydrocarbons.

INTRODUCTION

The metabolic activation of PAH to reactive electrophilic intermediates and their subsequent covalent binding to epidermal DNA are believed to be critical events in the initiation stage of mouse skin tumorigenesis (1-3). In fact, excellent correlations have been established between the varying carcinogenicities of many PAH and their covalent binding to DNA (4-6).

Fluorescence spectral data suggest that the metabolic activation of DMBA in rodent embryo cells (7-11) and mouse skin (12, 13) proceeds through the formation of a 3,4-diol-1,2-epoxide. Furthermore, recent evidence shows that, in these systems, both anti- and syn-3,4-diol-1,2-epoxide metabolites are significant contributors to the total binding of DMBA to DNA (14, 15), and that both adenine and guanine residues are major targets for modification (16). Dipple and coworkers (17) have suggested that the total binding to adenine correlates most closely with the biological activity of DMBA.

Structure-activity studies have proved useful in further understanding the mechanism of tumorigenesis by PAH, especially DMBA. For example, it has been shown that the introduction of fluorne atoms at various positions on the DMBA molecule can dramatically alter its biological activity (18-22). In a previous study which examined the tumor-initiating activity of a series of monofluoro-DMBA derivatives, activity was found to range from very weakly active to more active than the parent compound (22). The presence of a fluorine atom at carbon 10 of DMBA, surprisingly, increased tumor-initiating activity (22) and DNA binding capacity as compared to DMBA (23). An analysis of the epidermal DNA adducts formed from 10-F-DMBA showed marked increases in formation of adducts tentatively identified as the major anti-diol-epoxide-dGuo and dAdo adducts. Conversely, there was relatively little change in the absolute amount of syn-diol-epoxide adducts formed. The DNA adduct whose formation increased the greatest with 10-F-DMBA was the adduct tentatively identified as the major anti-diol-epoxide-dGuo derivative, suggesting that binding to this base might also be important for the biological activity of DMBA.

We have now examined, in detail, the formation of epidermal DNA adducts after topical application of 9-F-DMBA in SENCAR mice. Previous studies from our laboratories have shown that 9-F-DMBA is equipotent with DMBA for skin tumor-initiating activity (22). In this paper, we also present a comparison of the DNA adducts formed with DMBA, 9-F-DMBA, and 10-F-DMBA. The results demonstrate an excellent correlation between formation of a specific adduct (tentatively identified as the major anti-diol-epoxide-dAdo adduct) and skin tumor initiation by these PAH. In addition, data are presented to further support the hypothesis that binding to the adenine residues in DNA may be important for mouse skin tumor initiation by other PAH.

MATERIALS AND METHODS

Chemicals. DMBA was purchased from Eastman Kodak Co. (Rochester, NY), and [G-3H]DMBA (specific activity, 44 Ci/mmole) was acquired from Amersham Corp. (Arlington Heights, IL). 9-F-DMBA and 10-F-DMBA were generous gifts from Dr. M. S. Newman (Ohio State University, Columbus, OH), and [G-3H]9-F-DMBA (specific activity, 0.114 Ci/mmole) and [G-3H]10-F-DMBA (specific activity, 0.41
Cl/mmol) were prepared by the Midwest Research Institute (Kansas City, MO). 9-F-DMBA, 10-F-DMBA, [G-3H]9-F-DMBA, and [G-3H]10-F-DMBA were purified prior to use by preparative HPLC on a Dupont 8800 series HPLC equipped with a Zorbax octadecyl silicate column (21.2 mm × 25 cm). The column was maintained at ambient temperature with a flow rate of 5 ml/min. The methanol:water gradient system used consisted of a 30-min linear gradient from 50% methanol in water to 100% methanol. All hydrocarbons used in the present study were ≥ 98% pure as determined by HPLC.

Trans-4,5-dihydro-4,5-dihydroxybenzo(a)pyrene [trans-B(a)P-4,5-diol] and trans-9,10-dihydro-9,10-dihydroxybenzo(a)pyrene [trans-B(a)P-9,10-diol], used as UV markers in HPLC runs, were obtained from the National Cancer Institute, Chemical Carcinogen Repository, Bethesda, MD. Sephadex LH-20 was purchased from Pharmacia Fine Chemicals, Inc. (Piscataway, NJ). Servacel DHB was supplied by Accurate Chemical Co. (Westbury, NY). RNase A (EC 3.1.4.22) was obtained from Worthington Biochemical Co. (Freehold, NJ), while DNase I (bovine pancreas, EC 3.1.4.5), snake venom phosphodiesterase (Crotalus atrox, EC 3.1.5.1), and alkaline phosphatase (Escherichia coli, type II, EC 3.1.3.1) were obtained from Sigma Chemical Co., St. Louis, MO.

Covalent Binding to Mouse Epidermal DNA in Vivo. Individual groups of 10 female SENCAR mice (National Cancer Institute, Frederick, MD), aged 7 to 9 wk were used for each experimental group. The animals were shaved on the dorsal side 2 days prior to treatment. Mice received topical applications of various doses of tritium-labeled DMBA, 9-F-DMBA, or 10-F-DMBA. All animals were killed 24 h after treatment unless otherwise noted, and the isolation of DNA and quantitation of hydrocarbon binding followed procedures described by us previously (25). Binding is expressed as pmol of hydrocarbon covalently bound per mg of epidermal DNA.

Analysis of Hydrocarbon-DNA Adducts. DNA samples were hydrolyzed sequentially using DNase I, snake venom phosphodiesterase, and alkaline phosphatase as described previously (25). The DNA hydrolysates were subsequently separated using HPLC. Prior to injection into the HPLC, DNA hydrolysates were applied to a short column of Sephadex LH-20 (0.9 × 3 cm) and washed with water (10 ml) to remove unhydrolyzed DNA and unmodified deoxyribonucleosides. The less polar hydrocarbon-DNA adducts were then eluted with 5 to 10 ml of methanol. Some DNA samples were further manipulated by passing them through a dihydroboronate column of Servacel DHB (0.9 × 3 cm) using the procedure described by Sawicki et al. (17), allowing separation of syn- and anti-diol-epoxide adducts of DMBA, 9-F-DMBA, and 10-F-DMBA. We have previously used this procedure in the analysis of DMBA- and 10-F-DMBA-DNA adducts formed in mouse epidermis (23). The HPLC procedure used for separation of DMBA-, 9-F-DMBA-, and 10-F-DMBA-DNA adducts has been described elsewhere (23).

RESULTS

The total covalent binding of DMBA and 9-F-DMBA to mouse epidermal DNA was compared over a range of 10 to 100 nmol per mouse. The dose-response relationships observed in this range were very similar for both compounds with dose-dependent increases in covalent binding observed (Table 1). 9-F-DMBA was found to bind maximally to mouse epidermal DNA by 24 h after topical application and thereafter decrease. The levels of covalently bound material obtained at 12 and 72 h after topical application of 100 nmol of 9-F-DMBA were 17.3 ± 0.6 and 9.3 ± 0.3 pmol per mg of epidermal DNA, respectively. These values represent the mean ± SD of two separate experiments where groups of 10 mice were utilized for each time point. The 24-h value (Table 1) represents the mean ± SD of 6 experiments. These results are similar to recent experiments we have performed on the time course of DMBA binding to epidermal DNA. In previous work from our laboratories with 10-F-DMBA (23), we showed that this monofluorodervative bound more extensively to epidermal DNA than DMBA at all doses tested over a range of 4 to 200 nmol/mouse. Thus, the total covalent binding of both 9-F- and 10-F-DMBA cor-
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relates closely with their skin tumor-initiating activity compared with the parent hydrocarbon, DMBA.

A more detailed analysis of the covalently bound material derived from 9-F-DMBA was obtained by hydrolyzing the isolated DNA samples to deoxyribonucleosides and performing dihydroboronate (with Servacel DHB) chromatography as described in “Materials and Methods.” Fig. 1 shows the Servacel DHB chromatograms of DNA samples isolated from mice treated 24 h prior to sacrifice with 100 nmol of DMBA (Fig. 1a) and 9-F-DMBA (Fig. 1b). Also shown for comparative purposes is the Servacel DHB chromatogram of a representative DNA sample from animals treated with 100 nmol of 10-F-DMBA (Fig. 1c). The percentages of syn- (Peak I) and anti- (Peak II) diol-epoxide-DNA adducts derived from DMBA and 9-F-DMBA were significantly different. As noted previously (14, 23), the proportion of syn-diol-epoxide-DNA adducts from the 9-F-DMBA-treated mice was relatively high. In the representative chromatogram shown in Fig. 1 (a), 57% of the adducts eluted as syn-diol-epoxide adducts (Peak I). On the other hand, the proportion of syn-diol-epoxide-DNA adducts from the 9-F-DMBA-treated mice was only 24%. It should be noted that these results with 9-F-DMBA are similar to the results obtained with 10-F-DMBA as shown in Fig. 1c. The percentage of syn-diol-epoxide adducts with 10-F-DMBA was found to be 18%. Examination of selected DNA samples from DMBA-, 9-F-DMBA-, and 10-F-DMBA-treated mice by HPLC confirmed the marked difference in the percentage of syn- and anti-diol-epoxide adducts derived from DMBA compared with the two monofluoro derivatives (see below).

Fig. 2 shows the HPLC elution profiles of epidermal DNA samples obtained from mice treated with 100 nmol of DMBA (a), 9-F-DMBA (b), or 10-F-DMBA (c). The HPLC chromatograms shown in Fig. 2 were obtained from DNA samples that were optimally digested prior to chromatography. This was evidenced by the fact that 80.0 ± 3.5%, 77.0 ± 4.5%, and 82.0 ± 6.5% of the total radioactivity present in epidermal DNA samples (from DMBA-, 9-F-DMBA-, and 10-F-DMBA-treated mice, respectively) could be recovered in the methanol phases after LH-20 chromatography of digested DNA samples. The profiles in Fig. 2 were obtained from samples where 80% of the total radioactivity was present in the methanol phases. In agreement with previous studies using the mouse skin model (14, 23, 24, 26), 3 major DNA adducts are formed following topical applications of DMBA (Fig. 2, Peaks I, II, and III). Characterization of these adducts has shown that they arise through the reaction of the 3,4-diol-1,2-epoxides of DMBA with DNA (9–15). Peak II arises from the reaction of the syn-diastereomer with dAdo residues, while Peaks I and III are formed through the reaction of the anti-diastereomer with dGuo and dAdo residues, respectively (14–16). It should be pointed out that, in the present study, we have assumed that the dGuo and dAdo adducts derived from 9-F-DMBA and 10-F-DMBA elute from the HPLC column in the same order as the corresponding DMBA adducts. Analysis by HPLC of the syn- and anti-diol-epoxide-DNA adduct fractions from dihydroboronate chromatography (Fig. 1) for DMBA, 9-F-DMBA, and 10-F-DMBA samples confirmed these tentative assignments. In addition, preliminary experiments with DNA samples from primary mouse epidermal cells prelabeled with [3H]guanine and exposed to either [3H]DMBA or [3H]10-F-DMBA indicated that Peaks I/I' contain the 3H label, whereas Peaks II/II' and III/III' had little or no 14C label (23).

Fig. 2b shows the HPLC elution profile of DNA adducts obtained from mice treated with 100 nmol of 9-F-DMBA. Qualitatively, the chromatogram is very similar to that of the DNA adduct profile from DMBA-treated mice; however, the major peaks (I', II', and III') are significantly different with regard to the percentage they represent of the total radioactivity chromatographed. The percentage of 9-F-DMBA adduct Peak I' was dramatically increased as compared to DMBA adduct Peak I, while Peak II' represented significantly less of the total radioactivity chromatographed, compared to its DMBA counterpart (Peak II). Peak III' represented only slightly less of the total radioactivity chromatographed when compared to its DMBA counterpart (Peak III). Therefore, quantitatively Peak III'/III was formed in approximately equal amounts in mice treated with DMBA and 9-F-DMBA, respectively (see below). Fig. 2c shows the HPLC elution profile for a representative 10-F-DMBA sample. Interestingly, the percentages of the 3 major adducts derived from this monofluorodervative followed a pattern similar to 9-F-DMBA except that Peak III' represented a somewhat greater percentage of the total radioactivity chromatographed.

Table 2 summarizes the distribution of the 3 major adducts...
in DNA samples from DMBA-, 9-F-DMBA-, and 10-F-DMBA-treated mice (at the 100 nmol per mouse dose). In addition, the actual pmol per mg of DNA have been calculated and presented in Fig. 3. These values were obtained by taking the percentage shown for each peak in Table 2 and multiplying by the amount of material covalently bound at the 100 nmol/mouse dose for DMBA, 9-F-DMBA, and 10-F-DMBA expressed as pmol/mg of epidermal DNA. Values were obtained by multiplying the percentages shown for Peaks I/, II/, and III/ in Table 2 by the total covalent adducts bound at the 100 nmol/mouse dose for DMBA, 9-F-DMBA, and 10-F-DMBA (16.2 ± 2.4, 18.4 ± 2.4, and 52.3 ± 6.8 pmol/mg of epidermal DNA, respectively). Recovery of adducts after LH-20 in all samples was the same (see “Results”); thus, values in Fig. 3 have not been corrected and are based on total binding.

Table 2. DMBA-, 9-F-DMBA-, and 10-F-DMBA-DNA adduct distribution in DNA samples from mice treated with 100 nmol of the hydrocarbon

<table>
<thead>
<tr>
<th>DNA adduct peak</th>
<th>DMBA</th>
<th>9-F-DMBA</th>
<th>10-F-DMBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/ (anti-dGuo)</td>
<td>23.2 ± 3.7</td>
<td>48.3 ± 5.3</td>
<td>46.3 ± 1.0</td>
</tr>
<tr>
<td>II/ (syn-dAdo)</td>
<td>25.0 ± 2.0</td>
<td>11.0 ± 1.7</td>
<td>7.8 ± 0.2</td>
</tr>
<tr>
<td>III/ (anti-dAdo)</td>
<td>18.0 ± 0.9</td>
<td>14.1 ± 1.4</td>
<td>18.8 ± 0.4</td>
</tr>
</tbody>
</table>

* Values are calculated as the percentage each peak represents of the total radioactivity chromatographed.

DISCUSSION

In the present paper we have compared the formation of epidermal DNA adducts with DMBA, 9-F-DMBA, and 10-F-DMBA. A quantitative comparison of the 3 major DNA adducts formed from each hydrocarbon was made at a 100 nmol per mouse dose. Our previous dose-response experiments for skin tumor initiation (22) showed DMBA and 9-F-DMBA to be approximately equally active, whereas 10-F-DMBA was 2 to 3 times more active at this dose. The fluorine atom in these monofluoroderivatives is located in a position far removed from the bay-region of DMBA (22). In studies where fluorine is substituted for hydrogen in the bay-region benzo-ring of DMBA (i.e., positions 1, 2, 3, or 4) a dramatic inhibition of biological activity (18-22, 28), metabolism, and DNA adduct formation (29, 30) has been observed. These latter monofluoroderivatives have been useful in showing indirectly that bay-region diol-epoxide formation is important for the tumor-initiating activity of DMBA. However, they have not been useful in determining which specific DNA adduct(s) is important for DMBA tumor initiation. We chose to study several monofluoroderivatives of DMBA with either similar (9-F-DMBA) or greater (10-F-DMBA) skin tumor-initiating activity. It was felt that such derivatives might provide better clues regarding the specific DMBA-DNA adducts responsible for biological activity. This idea was further supported by the suggestion of Shiekh et al. (31) that fluorine substituted for hydrogen in positions 8, 9, 10, and 11 of DMBA could alter bay-region metabolism through electronic effects.

Recently, we demonstrated that the presence of a fluorine atom at position 10 of DMBA increased total covalent binding.
of this derivative to epidermal DNA compared with the parent hydrocarbon (DMBA) over a wide dose range (23). In addition, a dramatic reduction in the percentage of syn-diol-epoxide adducts was observed. With 10-F-DMBA, there was an increase in the absolute amounts of adducts tentatively identified as anti-3,4-diol-1,2-epoxide covalently bound to both guanine and adenine residues. In our present study, we compared DMBA, 9-F-DMBA, and 10-F-DMBA at a dose of 100 nmol per mouse. The results with 10-F-DMBA at this higher dose were similar to our previous results using 25 nmol per mouse (23). At the higher dose (100 nmol per mouse) there was a 6-fold increase in formation of Peak I/I' (tentatively identified as the major anti-diol-epoxide-dGuo adduct) with 10-F-DMBA compared to DMBA. In contrast, Peak II/II' (tentatively identified as the major syn-diol-epoxide-dAdo adduct) was present in approximately equal amounts. Peak III/III' (tentatively identified as the major anti-diol-epoxide-dAdo adduct) was present at about 3 times that in corresponding DMBA samples.

A surprising observation in our present study was the similarity in the DNA adduct profile between 9-F-DMBA and 10-F-DMBA, although 9-F-DMBA is approximately equipotent with DMBA for skin tumor initiation (22). With 9-F-DMBA there was also increased formation of Peak I/I' (Figs. 2 and 3; Table 2) to a level ~2.5 times that found in corresponding DMBA samples (8.5 versus 3.7 pmol per mg of DNA, respectively). Again, like 10-F-DMBA, there was a marked reduction in the percentage of Peak II/II' (tentatively identified as the major syn-diol-epoxide-dAdo adduct). This adduct was actually present at a level ~50% that found in corresponding DMBA samples (1.9 versus 4.0 pmol per mg of DNA, respectively). Interestingly, Peak III/III' (tentatively identified as the major anti-diol-epoxide-dAdo adduct) was present at a level essentially identical to that in corresponding DMBA samples. Our data, therefore, show that the level of only one of the 3 major adducts correlates closely with the biological activity of these 3 hydrocarbons on mouse skin, namely, Peak III/III' or the adduct we have tentatively identified as the major anti-diol-epoxide-dAdo (Fig. 3). If we extrapolate our previous dose-response data for tumor initiation with DMBA and 10-F-DMBA in SENCAR mice to allow comparison at 100 nmol per mouse (22), we find 10-F-DMBA to be ~2.4 times more active than DMBA and 9-F-DMBA. This difference is very close to the difference in formation of peak III/III' between DMBA (and 9-F-DMBA) and 10-F-DMBA (Table 2; Fig. 3). Given the difficulties of our studies, especially in terms of biological variations, these values are surprisingly close. The data also suggest that the presence of fluorosubstituents at carbons 9 or 10 of DMBA influences the interaction of the corresponding diol-epoxides with DNA. This can be seen with the large increase in binding of the anti-diol-epoxide with dGuo compared to dAdo residues. This latter point deserves further investigation.

Numerous investigations have attempted to determine the underlying mechanism of PAH tumor initiation in mouse skin as well as in other tissues. With benzo(a)pyrene, considerable information is available regarding the covalent binding of reactive metabolites with DNA. The major DNA adduct that is believed to be responsible for the tumor-initiating activity of B(a)P is (+)-anti-BPDE-dGuo (reviewed in Ref. 2). However, work from several laboratories has shown that (+)-anti-BPDE also binds to dAdo residues in epidermal DNA (32–34). Dipple and coworkers (14, 17) have suggested that the difference in potency between B(a)P and B(a)P for mouse skin tumor initiation correlates with their total extents of modification of dAdo residues in DNA. In light of this suggestion and with our data in this present paper suggesting a specific DMBA-dAdo adduct, we have compared the covalent binding of B(a)P and DMBA at a dose that gives an equivalent papilloma response in SENCAR mice (i.e., the same mice used in our present DNA binding study). This comparison is presented in Table 3 and is based on dose-response experiments from our laboratory for tumor initiation and DNA binding with both B(a)P and DMBA in SENCAR mice (Refs. 22, 23, 28, 33, and 35; Footnote 5). The dose of B(a)P giving a papilloma response similar to 4 nmol of DMBA is 284 nmol (or 71 times the dose of DMBA). The total covalent binding at these 2 doses is 17.7 and 2.2 pmol per mg of epidermal DNA for B(a)P and DMBA, respectively. We have calculated, in Table 3, the amount of the major dGuo adduct formed from the 2 hydrocarbons. In addition, we have calculated the amount of dAdo adducts formed from (+)-anti-BPDE at the 284-nmol dose of B(a)P compared with the amount of the major anti-diol-epoxide-dAdo adduct (Peak III; this study) formed from DMBA at the 4-nmol dose. Although there are 3 dAdo adducts formed from anti-BPDE in mouse epidermis, only 2 of these are formed from the (+)-enantiomer (33, 34). Since only the (+)-anti-BPDE possesses tumor-initiating activity on mouse skin (36) our calculations are restricted to the dAdo adducts formed with this enantiomer. Examination of these values shows that the extent of reaction with dAdo residues is quite similar and correlates closely with the magnitude of the papilloma response. The level of the major dGuo adduct, on the other hand, is found to be ~19 times greater with B(a)P than DMBA at the doses used in our comparison and clearly does not correlate with the papilloma response. Thus, although our present data, including the comparisons in Table 3, do not prove that dAdo in DNA is a critical target for B(a)P and DMBA, they do show a clear-cut quantitative correlation between the formation of specific hydrocarbon metabolite-dAdo adducts and skin tumor initiation by these hydrocarbons.

In conclusion, it should be stressed that there was a good

Table 3 Comparison of covalent binding of B(a)P and DMBA in SENCAR mouse epidermis at doses producing equivalent skin tumor initiation response

<table>
<thead>
<tr>
<th>Hydrocarbon</th>
<th>Papillomas/ mouse</th>
<th>% of mice with papillomas</th>
<th>Total covalent binding</th>
<th>Quantity of major dGuo adducts*</th>
<th>Quantity of critical dAdo adducts*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B(a)P (284)</td>
<td>5.4</td>
<td>100</td>
<td>17.70</td>
<td>11.58</td>
<td>0.36</td>
</tr>
<tr>
<td>DMBA (4)</td>
<td>5.4</td>
<td>100</td>
<td>2.20</td>
<td>0.60</td>
<td>0.40</td>
</tr>
</tbody>
</table>

* pmol/mg of epidermal DNA of the (+)-anti-BPDE-dGuo adduct for B(a)P and the anti-diol-epoxide-dGuo (Fig. 2, Peak I) adduct for DMBA. The value for B(a)P is again an extrapolation and is based on the observation that the relative proportion of the (+)-anti-BPDE-dGuo adduct changes very little as a function of the dose of B(a)P (33). The DMBA value was obtained from actual data on chromatographic runs of 4 nmol of DMBA-DNA adduct samples.

Numbers in parentheses, dose (nmol).

- J. DiGiovanni, unpublished data.
quantitative correlation between total adduct formation and tumor initiation with DMBA and its monofluoroderivatives. At present, we cannot rule out the possibility that it is the total number of DMBA-DNA adducts formed, rather than the specific adducts, which accounts for the different biological potencies. If the mechanism of skin tumor initiation is similar for different types of hydrocarbons (i.e., B(a)P versus DMBA), then the comparison between these 2 hydrocarbons in Table 3 suggests that total binding is less critical than the formation of specific DNA adducts. Further work will be necessary to answer these questions.

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Note Added in Proof

Additional experiments have now been completed examining the dose-response relationships (over a 4 to 100 nmol/mouse range) for formation of peaks I/II, II/III, and III/IV from DMBA-DNA. Comparison of the data presented in the current paper, DNA adduct IH/IH' was the only adduct present, we cannot rule out the possibility that it is the total quantitative correlation between total adduct formation and number of DMBA-DNA adducts formed, rather than the specific adducts, which accounts for the different biological potencies. If the mechanism of skin tumor initiation is similar for different types of hydrocarbons (i.e., B(a)P versus DMBA), then the comparison between these 2 hydrocarbons in Table 3 suggests that total binding is less critical than the formation of specific DNA adducts. Further work will be necessary to answer these questions.
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