Benzo(e)pyrene-induced Alterations in the Metabolic Activation of Benzo(a)pyrene and 7,12-Dimethylbenz(a)anthracene by Hamster Embryo Cells

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ABSTRACT

Benzo(e)pyrene (BeP) is a cocarcinogen with benzo(a)pyrene (BaP) and an anticarcinogen with 7,12-dimethylbenz(a)anthracene (DMBA) in mouse skin initiation-promotion assays (Slaga, T. J., Jecker, L., Bracken, W. M. and Weeks C. E. Cancer Res. 7: 51-59, 1979). We have investigated the effects of BeP on the metabolic activation of BaP and DMBA in early-passage cultures of Syrian hamster embryo cells. BeP had no effect on BaP-induced mutation frequencies in hamster embryo cell-mediated assays with V79 target cells. However, it inhibited the DMBA-induced mutagenesis by as much as 10-fold at the highest dose tested.

Low doses of BeP did not affect the total amount of BaP metabolized, but the proportion of water-soluble metabolites was reduced, and the proportions of trans-7,8-dihydro-7,8-dihydroxybenzo(a)pyrene and trans-9,10-dihydro-9,10-dihydroxybenzo(a)pyrene were increased. Higher doses decreased BaP metabolism and caused similar alterations in the metabolite profile. In cultures treated with trans-7,8-dihydro-7,8-dihydroxybenzo(a)pyrene, BeP greatly reduced the oxidative metabolism of this diol. BeP inhibited DMBA metabolism at all doses tested; the proportion of water-soluble metabolites formed was decreased, and the proportions of trans-8,9-dihydro-8,9-dihydroxy-7,12-dimethylbenz(a)anthracene and trans-3,4-dihydro-3,4-dihydroxy-7,12-dimethylbenz(a)anthracene were increased.

The results demonstrate that BeP is an effective inhibitor of the secondary oxidation of carcinogenic hydrocarbon diols required to convert diols which are proximate carcinogens to ultimate carcinogens such as diol-epoxides. The balance between (a) limited inhibition with consequent increase in total exposure to the ultimate carcinogenic form and (b) sufficient inhibition to reduce exposure to the ultimate carcinogenic form may determine whether BeP acts as a co- or anticarcinogen with a particular carcinogenic hydrocarbon.

INTRODUCTION

Polycyclic aromatic hydrocarbons are common environmental contaminants frequently formed by pyrolysis during incomplete combustion of fossil fuels. Since a large number of hydrocarbons with different chemical structures are formed, humans can potentially be exposed to a very complex mixture of these chemicals (6, 33). Some polycyclic hydrocarbons are potent carcinogens in rodent bioassays; others have only weak or no carcinogenic activity (for review, see Ref. 15). However, many of those with little or no carcinogenic activity either increase or decrease the carcinogenic activity of others (for reviews, see Refs. 13 and 14). VanDuuren and Goldschmidt (32) found that several weakly carcinogenic polycyclic aromatic hydrocarbons such as pyrene, fluoranthene, and BeP2 increase the carcinogenic activity of BaP when applied repeatedly to the backs of mice. The effects of various combinations of polycyclic hydrocarbons on initiation in the 2-stage system of mouse skin carcinogenesis have also been examined (12, 21, 29-31). Several cocarcinogenic hydrocarbons such as pyrene, fluoranthene, and BeP increase the skin tumor-initiating activity of BaP, but decrease the initiating activity of DMBA in mouse skin (30). Thus, the same hydrocarbons can have either co- or anticarcinogenic activities depending on the carcinogenic hydrocarbon being used as the initiator (12, 30). BeP can also be comutagenic with BaP in the microsomer-mediated Salmonella mutation assay (19).

The mechanism(s) of the cocarcinogenic and anticarcinogenic activity of hydrocarbons in mouse skin has not been established. It has been suggested that the inhibition of DMBA-induced initiation by some hydrocarbons such as dibenz(a,c)anthracene is due to induction of aryl hydrocarbon hydroxylase activity (12). However, BeP is a cocarcinogen and coinitiator when applied with BaP to mouse skin and a very poor inducer of mouse skin aryl hydrocarbon hydroxylase activity (1, 27).

Quantitation of the various metabolic pathways followed by hydrocarbons is difficult in mouse skin in vivo, and metabolism is usually measured in either epidermal homogenates (14) or cells in culture (19). The use of the latter allows uniform exposure of the cells, quantitation of the metabolites formed, and concurrent bioassays for transformation, mutation, or cytotoxicity. The metabolism of BaP and DMBA have been studied extensively in many cell culture systems (2, 9, 11, 17, 18, 22, 25). BaP is initially oxidized to epoxide intermediates, which are rapidly converted to phenols or quinones or metabolized to dihydrodiols by epoxide hydrolase. These oxygenated metabolites are then further oxidized to more polar derivatives through similar mechanisms or conjugated to polar substances such as glutathione, sulfate, or glucuronic acid (for reviews, see Refs. 25 and 26). The major metabolites recovered in early-passage hamster embryo (HE) cell cultures are free 7,8-diol and 9,10-diol and glucuronic acid conjugates of the 9- and 3-phenols (4, 5, 23). DMBA is initially oxidized to epoxide intermediates, which are rapidly converted to phenols or quinones or metabolized to dihydrodiols by epoxide hydrolase. These oxygenated metabolites are then further oxidized to more polar derivatives through similar mechanisms or conjugated to polar substances such as glutathione, sulfate, or glucuronic acid (for reviews, see Refs. 25 and 26). The major metabolites recovered in early-passage hamster embryo (HE) cell cultures are free 7,8-diol and 9,10-diol and glucuronic acid conjugates of the 9- and 3-phenols (4, 5, 23). DMBA is metabolized similarly in these cells, mainly to the 8,9-, 10,11-, and 3,4-diol forms and unidentified phenol glucuronides (3).

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The abbreviations used are: BeP, benzo(e)pyrene; BaP, benzo(a)pyrene; DMBA, 7,12-dimethylbenz(a)anthracene; BaP-7,8-diol, trans-7,8-dihydro-7,8-dihydroxybenzo(a)pyrene; 9-OH-BaP, 9-hydroxybenzo(a)pyrene; DMBA-8,9-diol, trans-8,9-dihydro-8,9-dihydroxy-7,12-dimethylbenz(a)anthracene; HPLC, high-performance liquid chromatography; 6-TG, 6-thioguanine; HE, hamster embryo.
To determine if BeP has co- and/or antimutagenic activity with BaP or DMBA in a HE cell-mediated mutation assay with V79 target cells (20). We also examined the effects of BeP on the metabolism of these hydrocarbons in HE cells in order to determine the relationship of BeP-induced changes in hydrocarbon metabolism to its effects on mutation induction.

MATERIALS AND METHODS

Cells. Primary cultures were prepared from 12- to 13-day-old Syrian (Golden) hamster embryos (LGV; Charles River Breeding Laboratories, Wilmington, MA, or Engle Laboratory Animals, Inc., Farmersburg, IN) as described previously (8). The V79-16 subline (10) of the V79 Chinese hamster lung cell line was used in the mutation experiments. This subline was routinely monitored for Mycoplasma contamination (7) and was shown to be negative. Unless otherwise specified, all cells were grown without antibiotics in Eagle's minimal essential medium (Auto-Pow; Flow Laboratories, Rockville, MD) containing an additional mixture of vitamins as formulated for Eagle's basal medium and supplemented with 10% fetal bovine serum (Reheis Chemical Co., Chicago, IL).

Cell-mediated Mutation Assay. The procedure for the cell-mediated mutation assay based on that of Huberman and Sachs (20), has been described in detail (10). The medium used throughout was Eagle's basal medium (Grand Island Biological Co., Grand Island, NY) supplemented with 10% fetal bovine serum. Second-passage HE cells were irradiated in suspension (2500 rads from a 60Co source) and seeded at a density of 2.3 x 10^6 cells/25-sq cm flask (Falcon Plastics, Oxnard, CA). One day later, 2.7 x 10^5 V79-16 cells were added to each flask and, after 2 hr, BeP (at the concentrations indicated) or acetone was added. Five min later, BaP or DMBA (from acetone stock solutions, 1 mg/ml) was added to a final concentration of 0.5 or 0.1 ng/ml, respectively; acetone was added to control flasks to a final concentration of 0.05%. The V79 cells were harvested after 24 or 48 hr, and hydrocarbon-induced toxicity was determined by seeding 100 V79 cells into each of ten 60-mm plates and staining the colonies 7 days later. For determining mutation frequencies, 1.2 x 10^4 treated V79 cells first were also seeded into a 75-sq cm flask and replated 4 days later. To determine viability, 100 cells were seeded into 60-mm plates and the colonies stained 7 days later; to determine the number of mutants, 4 x 10^5 cells were seeded into twenty-five 60-mm plates in medium containing 6-TG (final concentration, 0.1 mM) (4-day expression time), the cultures were refed with 6-TG after 4 days and stained 7 days later.

Hydrocarbon Metabolism. Confluent cultures of second- or third-passage HE cells in 25-sq cm flasks containing 10 ml medium were treated with BeP (from acetone stock, 1 mg/ml; final concentration as indicated) or acetone. Five min later, either [G-3H]BaP, [G-3H]DMBA, or [G-3H]BaP-7,8-diol was added to give the concentration stated in the chart legends. The [G-3H]BaP (specific activity, 21 to 26 Ci/mmol; Amersham Corp., Arlington Heights, IL) was diluted with unlabeled BaP (Gold Label; Aldrich Chemical Co., Milwaukee, Wl) to a specific activity of 5 Ci/mmol. The [G-3H]DMBA (specific activity, 10 to 28 Ci/mmol) was obtained from Amersham, and the [H]BaP-7,8-diol (specific activity, 381 mCi/mmol) was from the Radiochemical Repository, Division of Cancer Cause and Prevention, National Cancer Institute. After incubation, medium samples were removed, extracted according to a 2-step chloroform:methanol:water procedure (5), and the amount of radioactivity in aliquots of each phase was determined by liquid scintillation counting. To measure the formation of hydrocarbon-glucuronides, medium samples were incubated with 2000 Fishman units of bovine liver /3-glucuronidase prior to extraction, as described previously (4, 5). The chloroform phases were analyzed by HPLC on an Altex Model 312 chromatograph equipped with a 4.6-mm x 25-cm Ultrasphere octadecyl silane (5 /¿m) reverse-phase column (Altex Scientific Inc., Berkeley, CA). BeP samples were eluted at 1 ml/min for 40 min with a linear methanol:water gradient from 11.3 to 17.3 followed by 10 min at 17.3 (4). One hundred ten fractions (0.5 ml) were collected, and radioactivity was determined by liquid scintillation counting. Reference standards of BaP metabolites were provided by the Chemical Repository, Carcinogenesis Research Program, National Cancer Institute. DMBA samples were chromatographed on the same column eluted with a methanol:water gradient from 1:1 to 7:3 in 20 min, 7:3 for 10 min, 7:3 to 9:1 in 20 min, and 9:1 for 30 min. One hundred and sixty 0.5-min fractions were collected. Reference standards were provided by Dr. J. DiGiovanni and Dr. A. Dipple. For each experiment, a flask containing medium and [3H]-hydrocarbon but no cells was included as a control. The amount of original material recovered at the end of the incubation period, determined as described above, was never less than [3H]BaP, 96%; [3H]BaP-7,8-diol, 98%; and [3H]DMBA, 92%.

RESULTS

Effects of BeP on Mutation Induction. The effects of BeP on the frequencies of 6-TG-resistant mutants induced in V79 target cells by BaP and DMBA were measured in HE cell-mediated assays. At BeP to BaP ratios of 2:1, BeP had no effect on BaP-induced toxicity or mutation frequencies; after a 24-hr treatment, the mutation frequencies induced in the absence or presence of BeP were 35 to 39 mutants/10^6 V79 cells (Table 1). BeP markedly inhibited toxicity and mutagenicity induced by DMBA (Table 1).
Effect of BeP on BaP Metabolism. The metabolite profiles of 
$[^3H]$BaP after a 24-hr exposure to HE cells in the absence and presence of BeP are shown in Chart 1. In the samples from cells treated with BaP alone or with BaP plus BeP, the major organic solvent-extractable metabolites were the 9,10-diol, 7,8-diol, and 9-OH-BaP. The BaP plus BeP sample contained significantly more of both diols than did the BaP alone sample. $\beta$-Glucuronidase treatment released similar amounts of 9- and 3-0H-BaP from both groups.

The amounts of BaP metabolites formed in the control and BeP-treated groups at 24, 48, and 72 hr are shown in Table 3. At all time points, the total amounts of BaP metabolized by the 2 groups was not significantly different, but the amount metabolized to water-soluble derivatives was significantly lower in the BeP-treated group at all time points. This decrease in water-soluble metabolites in the BeP-treated groups was observed in medium samples with and without $\beta$-glucuronidase treatment. It was not due to inhibition of the formation of phenol glucuronides by BeP, for the proportion of metabolites released by $\beta$-glucuronidase treatment as 9 and 3-0H-BaP was identical in the BeP and control groups. BeP treatment resulted in an increase in the proportions of BaP-9,10-diol and BaP-7,8-diol at all time points. In the BeP-treated groups, the proportions of both diols were more than doubled as compared to the control at both 24 and 48 hr, and the concentration of BaP-9,10-diol was more than 5 times greater at 72 hr. In contrast, BeP treatment resulted in only a slight increase in free 9-OH-BaP at 24 hr and had no effect on either free or glucuronic acid-conjugated phenols at 48 or 72 hr.

As shown in Chart 2, there were no qualitative alterations in the HPLC profiles of the organic solvent-extractable BaP metabolites with increased concentrations of BeP; BaP-9,10-diol, BaP-7,8-diol, and 9-OH-BaP were the major metabolites in all samples. However, there were changes in the proportions of the BaP metabolites formed and decreases in the amounts of BaP metabolized (Table 4). The proportion of BaP converted to watersoluble metabolites decreased with increasing concentrations of BeP, and the proportion of BaP metabolized to BaP-9,10-diol, BaP-7,8-diol, and 9-OH-BaP increased. Thus, the proportion of the BaP metabolites recovered as primary oxidation products increased as the concentration of BeP increased. In the case of BaP-7,8-diol, this represented an actual increase in the amount present in the culture medium from 12.5 ng/ml in the control group to 15.7 ng/ml in the group treated with 1 $\mu$g BeP/ml. At higher BeP doses, the increase in the percentage of BaP metabolized to BaP-7,8-diol was essentially equal to the decrease in overall metabolism, so that the level of BaP-7,8-diol remained approximately constant, 13.7 ng/ml medium in the 2-$\mu$g/ml BeP group and 12.7 ng/ml in the 5-$\mu$g/ml BeP group.

To determine how BeP treatment alters the metabolism of BaP-7,8-diol, cells were exposed to $[^3H]$BaP-7,8-diol in the presence of BeP and the metabolites analyzed after 24 hr (Chart 3). The HPLC elution profiles contained only 3 major peaks, unmetabolized $[^3H]$BaP-7,8-diol and 2 polar metabolite peaks in Fractions 15 to 21 and 22 to 25. These polar metabolites eluted in positions similar to tetrins prepared from BaP-7,8-diol-9,10-epoxide. With increased concentrations of BeP, there were no qualitative changes in the metabolite elution profiles, but there were quantitative changes (Table 5). Metabolism of BaP-7,8-diol decreased from 85% metabolized in the control group to 26.8% metabolized in the 2-$\mu$g BeP/ml group. Both oxidation of BaP-7,8-diol to more polar derivatives and conjugation of BaP-7,8-diol and its derivatives to glucuronic acid were decreased. However, the decrease in oxidation of BaP-7,8-diol was proportionally much greater than the decrease in glucuronidation.

Effect of BeP on DMBA Metabolism. The effects of BeP on the metabolism of $[^3H]$DMBA are shown in Chart 4. In the absence of BeP, a large proportion of the DMBA was metabolized to very polar metabolites that eluted prior to Fraction 40. In the group treated with 0.3 $\mu$g BeP/ml, DMBA-8,9-diol was the major metabolite, and a number of the more polar metabolite peaks were absent. A peak coeluting with a marker of DMBA-3,4-diol was also present in this sample. In the groups exposed to 1.0 or 1.5 $\mu$g BeP/ml, the amount of DMBA remaining increased, and the major metabolite peaks were DMBA-8,9-diol, DMBA-3,4-diol, and DMBA phenols. Thus, as the concentration of BeP increased, the proportion of DMBA metabolized to polar derivatives decreased, and the proportion metabolized to DMBA-
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Chart 1. BaP metabolism in the absence (A) and presence (B) of BeP. Confluent cultures of HE cells were treated with 1.0 μg BeP/ml medium (B) or 0.1% acetone (A), and 5 min later, 0.5 μg [3H]BaP (specific activity, 1 Ci/mmol)/ml medium was added. After 24 hr of exposure, the medium was removed, and a 0.2-ml aliquot was extracted with chloroform:methanol, as described in “Materials and Methods.” An identical aliquot was treated with 2000 units of β-glucuronidase at 37° for 1 hr prior to extraction. The chloroform extracts were analyzed by HPLC, as described in “Materials and Methods.” The elution positions of BaP metabolite reference standards are shown at the top. D, β-glucuronidase-treated medium sample; •, medium sample with no enzyme treatment.

8,9-diol, DMBA-3,4-diol, and DMBA phenols increased.

The amounts of the major DMBA metabolites formed after 24 hr are shown in Table 6. A dose of only 0.3 μg BeP/ml caused some inhibition of DMBA metabolism, and 1.5 μg BeP/ml reduced the amount of DMBA metabolized to 57% of that of the control group. The major differences in DMBA metabolites in the BeP-treated groups compared to the control were the decrease in water-soluble metabolites and the increase in dihydrodiols. The formation of DMBA-3,4-diol was not detected in the absence of BeP at either 24 hr (Table 6) or 48 hr (Chart 4), but this diol was a major metabolite in all BeP-treated groups at both time points. In addition, the percentage of DMBA metabolized to DMBA-8,9-diol in the BeP-treated groups was more than twice that of the control.

DISCUSSION

To investigate the molecular mechanisms by which exposure to multiple hydrocarbons might alter the induction of biological effects, we examined the effects of BeP on mutation induction by BaP and DMBA in a HE cell-mediated assay and the effects of BeP on the metabolism of these carcinogenic hydrocarbons. One limitation of the mutation assays was that the total dose of hydrocarbon which could be used was about 2 μg/ml, for higher doses were toxic for the V79 cells in either the presence or absence of the HE activator cells and regardless of the structures of the hydrocarbons. The reason for this nonspecific hydrocarbon-induced cytotoxicity is not known, but it limited to 2:1 the maximum ratio of BeP to BaP which could be used. Because of the higher mutagenic activity of DMBA in this system, ratios of
BeP to DMBA as high as 15:1 could be used. At the 2:1 ratio, BeP had no significant effect on the frequency of BaP-induced mutations in the cell-mediated assay after 24 hr of exposure. The mutation frequency was reduced by about 35% by BeP treatment after 48 hr (data not shown). BeP reduced mutation induction by DMBA by 10-fold at the highest ratio of BeP to DMBA tested (15:1). Because of the limitations on the total amount of hydrocarbon which could be used, it was not possible to determine if the differences in the effects of BeP on BaP- and DMBA-induced mutation frequencies was due to the different ratios of hydrocarbons used.

Similar difficulties have been encountered in studies of the co- and anticarcinogenic activities of BeP in mouse skin, for the dose required for initiation by BaP was 20 times higher than that for DMBA (12, 30). BeP was a weak cocarcinogen with BaP, with a 2:1 ratio of BeP to BaP producing a slight increase in papilloma formation from a relative value of 100 ± 12% (S.D.) for the control to 130 ± 13% for the BeP-treated group. A group treated at a 1:1 ratio was also 130% of that of the control (12). On the other hand, BeP decreased the initiating activity of DMBA in mouse skin to 16% of that of the control when applied at BeP to DMBA ratios of 20:1 (12) or 40:1 (30). Thus, the cell-mediated mutation assay results with BeP closely paralleled the results obtained with BeP in the initiation promotion assays in vivo.

BeP treatment had different effects on the total metabolism of BaP and DMBA by the HE cell cultures. At a 2:1 ratio, BeP had no effect on the total metabolism of BaP at either 24, 48, or 72 hr. It did cause a decrease at high ratios, but even at a 5:1 ratio the decrease was only 40%. MacLeod et al. (24) reported an inhibition of BaP metabolism by BeP at a 40:1 dose ratio of BeP to BaP. Thus, BeP can inhibit total BaP metabolism but not at the doses used in the mutation assays. With DMBA, BeP caused only a slight inhibition of metabolism at a 3:1 ratio but more than 50% inhibition of BaP metabolism by BeP at a 40:1 dose ratio of BeP to DMBA (12). Because of the limitations on the total amount of hydrocarbon which could be used, it was not possible to determine if the differences in the effects of BeP on BaP- and DMBA-induced mutation frequencies was due to the different ratios of hydrocarbons used.

### Table 3
Effect of BeP on BaP metabolism

<table>
<thead>
<tr>
<th>Treatment protocol</th>
<th>Total BaP metabolism (% metabolized)</th>
<th>% of BaP metabolites formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (hr)</td>
<td>BeP</td>
<td>9,10-diol</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
<td>63.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>60.2 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>65.6 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>61.5 ± 1.8</td>
</tr>
<tr>
<td>48</td>
<td>-</td>
<td>88.3 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>88.1 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>88.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>88.6 ± 0.3</td>
</tr>
<tr>
<td>72</td>
<td>-</td>
<td>94.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>94.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>95.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>93.8 ± 0.7</td>
</tr>
</tbody>
</table>

a Average ± S.D. of 3 flasks.

### Table 4
Effect of dose of BeP on BaP metabolism

<table>
<thead>
<tr>
<th>BeP (ug/ml)</th>
<th>Total BaP metabolism (% metabolized)</th>
<th>% of BaP metabolites formed</th>
</tr>
</thead>
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<tr>
<td>0.0</td>
<td>75.7 ± 3.2</td>
<td>66.6 ± 6.8</td>
</tr>
<tr>
<td>1.0</td>
<td>73.2 ± 2.1</td>
<td>63.5 ± 1.1</td>
</tr>
<tr>
<td>2.0</td>
<td>53.0 ± 6.0</td>
<td>61.0 ± 5.0</td>
</tr>
<tr>
<td>5.0</td>
<td>46.1 ± 4.6</td>
<td>56.5 ± 3.8</td>
</tr>
</tbody>
</table>

a Average ± S.D. of at least 5 flasks for all results, unless noted otherwise.
b Average ± S.D. of 3 flasks for this line only.
c ND, not determined.

### Table 5
Effect of BeP on BaP-7,8-diol metabolism

<table>
<thead>
<tr>
<th>BeP (ug/ml)</th>
<th>β-Glucuronidase (IU/ml)</th>
<th>H2O-soluble metabolite</th>
<th>Unknown 1</th>
<th>Unknown 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>15.0</td>
<td>61.9</td>
<td>17.2</td>
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<td>0</td>
<td>+</td>
<td>30.0</td>
<td>30.0</td>
<td>29.6</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>73.3</td>
<td>19.4</td>
<td>4.9</td>
</tr>
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<td>2</td>
<td>+</td>
<td>83.5</td>
<td>7.4</td>
<td>6.3</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>79.7</td>
<td>15.5</td>
<td>3.4</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>81.9</td>
<td>5.1</td>
<td>4.4</td>
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in cells and in mouse skin (12). Our results indicate that BeP is not acting by inducing increased metabolism of the parent hydrocarbon, for no increase in either BaP or DMBA metabolism was observed. This is in agreement with the low activity of BeP as an inducer of aryl hydrocarbon hydroxylase activity in mouse skin (1, 27).

The major effect of BeP on the metabolism of BaP was the alteration of the proportion of the various classes of metabolites formed. At all time points, there was a decrease in the percentage of the BaP metabolites that were water soluble and an increase in the proportion of the BaP-7,8-diol and the BaP-9,10-diol.
Table 6

Effect of BeP on DMBA metabolism

<table>
<thead>
<tr>
<th>BeP (μg/ml)</th>
<th>% of DMBA metabolized</th>
<th>H₂O soluble</th>
<th>8,9-diol</th>
<th>3,4-diol</th>
<th>7-Hydroxymethyl Phenols</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98.4 ± 2.4</td>
<td>6.5 ± 2.8</td>
<td>8.3 ± 2.8</td>
<td>Not detectable</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>0.3</td>
<td>89.1 ± 3.9</td>
<td>47.2 ± 1.8</td>
<td>22.8 ± 3.0</td>
<td>5.7 ± 0.9</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>1.5</td>
<td>56.5 ± 2.9</td>
<td>42.7 ± 1.7</td>
<td>21.4 ± 4.3</td>
<td>6.2 ± 1.2</td>
<td>3.4 ± 0.6</td>
</tr>
</tbody>
</table>

* Average ± S.D. of 3 flasks.

The present results demonstrated that BeP may increase the proportion of DMBA metabolized to water-soluble metabolites and decreased tetroli formation to very low levels (see Chart 3). It also had an effect on DMBA metabolism, as the dose of BeP increased, the proportion of DMBA metabolized to water-soluble derivatives decreased, and the proportions of DMBA-8,9-diol and DMBA-3,4-diol increased. The shift in DMBA metabolite patterns is clearly illustrated in Chart 4, which shows that the control group had a large number of very polar metabolites eluting prior to DMBA-8,9-diol and essentially no DMBA-3,4-diol, whereas in the BeP-treated groups these polar metabolites were absent, and the 3,4-diol peak was evident. These BeP-induced alterations in BaP and DMBA metabolism could involve either competition for enzyme sites or could be due to a shift in the pattern of cytochrome P-450 isozymes. The present results would not distinguish between these possibilities nor would they establish whether the changes induced were caused by BeP itself or a metabolite(s) of BeP.

The ability of BeP to act as a co- or anti-initiating agent with various hydrocarbons may be related to both the ratio of BeP to hydrocarbon and the relative ability of BeP to inhibit the secondary oxidation of metabolites of that hydrocarbon. Our results with DMBA and BaP demonstrate that BeP may increase the proportions of the proximate carcinogenic diols, BaP-7,8-diol or DMBA-3,4-diol (for reviews, see Refs. 26 and 28), formed from the parent hydrocarbon. The effect of BeP on the subsequent activation of these diols to diol-epoxides may alter the time course and ultimate level of exposure to the ultimate carcinogenic form. In the case of BeP, the level of BaP-7,8-diol was higher in the BeP-treated group than in the control group at both 24 and 48 hr, but the actual amount present decreased in both groups by 72 hr (Table 3). However, in the case of DMBA, the 3,4-diol was undetectable in the control group at 24 and 48 hr but was present in the BeP-treated groups at both times (Table 6). BeP may inhibit the oxidation of this diol so effectively that it reduces the total amount of DMBA diol-epoxide formed. Thus, the ability of BeP to enhance or inhibit the induction of biological effects by carcinogenic hydrocarbons may depend on its ability to inhibit the conversion of the proximate carcinogenic diol of that hydrocarbon to a diol-epoxide.

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REFERENCES


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Benzo(e)pyrene-induced Alterations in the Metabolic Activation of Benzo(a)pyrene and 7,12-Dimethylbenz(a)anthracene by Hamster Embryo Cells

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