Benzo(a)pyrene:DNA Adduct Formation in Normal Human Mammary Epithelial Cell Cultures and the Human Mammary Carcinoma T47D Cell Line

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ABSTRACT

The benzo(a)pyrene (BaP):DNA adducts formed in normal human mammary epithelial cell cultures and the human mammary carcinoma T47D cell line were analyzed by chromatography and acid hydrolysis of the BaP:deoxyribonucleoside adducts to BaP:purine and BaP:tetraols. Human mammary epithelial cell cultures and human mammary carcinoma T47D cells were exposed to [3H]BaP for 24 h, and the levels of binding were 81 and 182 pmol BaP/mg DNA in normal and T47D cultures, respectively. Analysis of BaP:deoxyribonucleoside adducts resolved by immobilized boronate chromatography and reverse-phase high-performance liquid chromatography demonstrated the presence of three BaP:deoxyribonucleoside adducts in both cell lines: M2, M1, and M2. M2 contained cis-vicinal hydroxy groups, a configuration which would result from reaction of 7,8-ba-dihydroy-9a,10a-epoxy-7,8,9,10-tetrahydroBaP (anti-BaPDE) with DNA. M2 was identified as (+)-anti-BaPDE:deoxyguanosine (dGuo) for its cochromatography with [14C]-(+)-anti-BaPDE:dGuo marker, the BaP:purine hydrolysis product of M2 cochromatographed with [14C]-(+)-anti-BaPDE:dGuo, and the tetraol hydrolysis products cochromatographed with (±)-anti-BaPDE:tetraols. M1 was identified as (−)-anti-BaPDE:dGuo for M1 eluted in the same relative position as (−)-anti-BaPDE:dGuo marker, the BaP:purine hydrolysis product of M1 cochromatographed with [14C]-(−)-anti-BaPDE:dGuo, and the tetraol hydrolysis products cochromatographed with (−)-anti-BaPDE:tetraols. Thus, both adducts that bound to the immobilized boronate column were formed from (±)-anti-BaPDE. One major adduct that did not contain cis-vicinal hydroxy groups, M2, was detected in both cell types. M2 was formed from (±)-7,8-ba-dihydroy-9a,10a-epoxy-7,8,9,10-tetrahydroBaP (syn-BaPDE) as M2 eluted in the same relative position as syn-BaPDE:dGuo adduct marker and the tetraol hydrolysis products of M2 cochromatographed with tetraols formed from (±)-syn-BaPDE. The isolation of the individual BaP:DNA adducts followed by acid hydrolysis allowed the identification of the BaP:DNA adducts formed in human mammary cell cultures and demonstrated the presence of (−)-anti-BaPDE:dGuo. Thus, this work provides the first evidence, other than chromatography, that (−)-anti-BaPDE is formed in cell systems and reacts with DNA in cells to form (−)-anti-BaPDE:dGuo. The similarity of the BaP:deoxyribonucleoside adduct profiles in normal mammary epithelial cells and the human mammary carcinoma T47D cell line suggests that the T47D cell line is an excellent model for studies on the metabolic activation of polycyclic aromatic hydrocarbons to DNA binding intermediates in human mammary cells.

INTRODUCTION

Polycyclic aromatic hydrocarbons, such as BaP, are widespread environmental pollutants and many are potent carcinogens in the mammary tissue of rodents (1–5). BaP is metabolized in human mammary epithelial and fibroblastic cells to both water-soluble and organic solvent-soluble metabolites, including K-region and non-K-region dihydiolids: the 9,10-dihydrodiol is the organosoluble metabolite present in the greatest amount (6–10). We have recently compared BaP metabolism in normal human mammary epithelial cells and human mammary tumor cell lines (11–13). Both human mammary epithelial and human mammary carcinoma T47D cells have a high capacity for BaP metabolism. A large proportion of metabolites formed in both cells were water soluble. Further characterization of the metabolites formed in the T47D cells indicated that 30% were organic solvent extractable tetraols, dihydriols, quinones, and phenols. Water-soluble metabolites included sulfates (25%) and glutathione conjugates (35%), but only negligible amounts of glucuronides were detected.

In cells in culture and in vivo BaP can undergo metabolic activation to form an ultimate carcinogenic and mutagenic metabolite, BaPDE, reviewed in Refs. 14–16. BaPDE exists as a pair of diastereomers: anti-BaPDE (benzylic hydroxyl and epoxide on the opposite face of the plane of the molecule) and syn-BaPDE (benzylic hydroxyl and epoxide on the same face of the plane of the molecule). Each diastereomer consists of a pair of enantiomers: (+) and (−). BaP metabolism and DNA binding have been studied in a number of animal and human model systems. The major BaP:DNA adduct formed in rodent cell systems is (±)-anti-BaPDE:dGuo (reviewed in Refs. 14 and 16); smaller amounts of other BaP:deoxyribonucleoside adducts, including (−)-anti-BaPDE:dGuo, have been reported (17–22). The structural assignments of these adducts were accomplished by comparison with known BaP:deoxyribonucleoside adduct standards. However, we have recently used acid hydrolysis techniques to convert BaP:deoxyribonucleoside adducts to BaP:purine adducts and BaP:tetraols, and have shown that the BaP:DNA adduct peak usually assigned as (−)-anti-BaPDE:dGuo based upon its elution position actually results from a different unidentified BaP metabolite in early passage Wistar rat embryo cell cultures (23). BaP:DNA adduct formation has been less extensively studied in mammmary cell culture systems. Phillips et al. (24) detected the presence of seven BaP:DNA adducts in primary cultures of Wistar rat mammary cells (23). BaP:DNA adduct formation has been less extensively studied in mammmary cell culture systems. Phillips et al. (24) detected the presence of seven BaP:DNA adducts in primary cultures of Wistar rat mammary cells: none of these adducts were formed from (±)-anti-BaPDE. In contrast, MacNicoll et al. (6), Stampfer et al. (8), and Bartley et al. (9) have shown by chromatography with known standards that BaP is activated to DNA binding metabolites through the formation of anti-BaPDE in human mammary epithelial and fibroblastic cells.

In this investigation we have analyzed the BaP:deoxyribo-
nucleoside adducts formed in human mammary epithelial cells and the human mammary carcinoma T47D cell line using acid hydrolysis techniques to characterize the structure of the adducts formed and to compare BaP:deoxyribonucleoside adduct formation in normal human epithelial mammary cell cultures and the human mammary carcinoma cell line T47D.

MATERIALS AND METHODS

Cell Cultures. The human mammary epithelial cells were obtained from Dr. Charles M. McGrath, Michigan Cancer Foundation, Detroit, MI, and were grown in 75-cm² culture flasks with Dulbecco's plus Ham's F-12 medium, 1:1 (Grand Island Biological Co., Grand Island, NY) containing 30% horse serum (Grand Island Biological Co.), 100 ng chola enterotoxin per ml medium, 0.5 µg hydrocortisol per ml medium, 10 µg insulin per ml medium, 100 µg penicillin per ml medium, and 100 µg streptomycin per ml medium.

The human mammary carcinoma T47D cells were obtained from the National Cancer Institute Mammary Tumor Cell Repository through E. G. & G. Mason Research Institute, Rockville, MD, and were grown in 100-mm Petri dishes with RPMI 1640 (Grand Island Biological Co.) with 10% fetal bovine serum (Grand Island Biological Co.), 10 µg insulin per ml medium, 50 units penicillin per ml medium, and 50 µg streptomycin per ml medium.

Preparation of [3H]BaP-DNA Adducts. Human mammary epithelial cells were grown to 90–95% confluence in 100-mm Petri dishes. [3H]BaP (Aldrich Chemical Co., Milwaukee, WI) to a specific activity of 6 Ci/mmoll and was added to the cultures at a final concentration of 1 µg/ml medium (4 µM). After 24 h, the cells were harvested with trypsin:Versene (25) at —8°C. Human mammary carcinoma T47D cells were grown to 90–95% confluence in 100-mm Petri dishes. [3H]BaP (Amersham) was diluted with BaP (Aldrich Chemical Co.) to a specific activity of 6 Ci/mmoll and was added to the cultures at a final concentration of 4 µM for 24 h. The cells were then harvested with trypsin:Versene (25) and stored at —80°C.

The DNA was isolated from the human mammary epithelial cell pellet by a CsSO₄:dimethyl sulfoxide gradient procedure as described previously (26). The DNA was dialyzed against 1 mm EDTA, 12 h, shell frozen, and lyophilized. The sample was then reconstituted with 10% sodium dodecyl sulfate stock and 1 mM NaCl. Cold 95% ethanol was added and the sample was stored at —20°C for 12 h to ensure complete precipitation. The DNA was then redissolved in water.

The DNA was isolated from the human mammary carcinoma T47D cell pellets by homogenization in Tris buffer (10 mM Tris-HCl:1 mM EDTA:1% sodium dodecyl sulfate, pH 8) and incubated at 37°C for 30 min with proteinase K (2 mg/ml) as described previously (21). The mixture was extracted with chloroform:isoamyl alcohol (24:1, v/v) and the DNA was precipitated with 0.1 volume of 2 M NaCl and 2 volumes of 95% ethanol. The DNA was dissolved in 0.01 M Tris, pH 7.2, and treated with RNase A (0.1 mg/ml) for 20 min at 37°C, followed by proteinase K for 10 min at 37°C, extracted with chloroform:isoamyl alcohol, and precipitated with ethanol. The enzyme treatments, chloroform:isoamyl alcohol extraction, and ethanol precipitation was repeated. The DNA was then treated with RNase A, exonuclease III, and proteinase K, followed by ethanol precipitation. The DNA was redissolved in 0.01 M Tris, pH 7.2, and precipitated with ethanol, rinsed with ethanol, and redissolved in 0.01 M Tris, pH 7.2.

The DNA was enzymatically degraded to deoxyribonucleosides (27). The DNA hydrolysates were applied to Sep-Pak C₈ cartridges (Waters Associates, Milford, MA). Unreacted deoxyribonucleosides were eluted in 20 ml water and the BaP:deoxyribonucleoside adducts were eluted in methanol by washing the Sep-Pak with 1 ml methanol:water (40:60), followed by three 2-ml fractions of methanol. Aliquots of each methanol fraction were removed and the radioactivity in each aliquot was determined by liquid scintillation counting using ACS (Amersham). The methanol fractions containing radioactivity were pooled and the BaP:deoxyribonucleoside adducts were analyzed.

Immobilized Boronate Chromatography. The BaP:deoxyribonucleosides that contained cis-cisoidal hydroxyl groups were separated from the other BaP:deoxyribonucleoside adducts by chromatography on a column of [1N-1N-[m-(dihydroxyboryl) phenyl]succinamylaminooethyl] cellulose as described previously (21). The BaP:deoxyribonucleoside adducts not containing cis-cisoidal hydroxyl groups were eluted with 1 M morpholine, pH 9, buffer. The BaP:deoxyribonucleoside adducts containing cis-cisoidal hydroxyl groups were then released from the column with the morpholine buffer containing 10% sorbitol. The [3H]BaP:deoxyribonucleoside adducts in each buffer fraction were combined and concentrated by chromatography on a Sep-Pak C₈ cartridge. After elution of the buffer components with water, the BaP:deoxyribonucleosides were eluted from the Sep-Pak cartridges with methanol and the individual BaP:deoxyribonucleoside adducts were analyzed by reverse-phase HPLC.

HPLC Analysis of BaP:Deoxyribonucleoside Adducts. The BaP:deoxyribonucleoside adducts were chromatographed by HPLC on a 25 cm x 4.6-mm Ultrasphere octyl reverse-phase column (Beckman Instruments, Inc., St. Louis, MO). The column was eluted with methanol:water (46:54) for 34 min at a flow rate of 1.0 ml/min, then for 10 min with a linear gradient of methanol:water (46:54 to 55:45), followed by 24 min with methanol:water (55:45). Fifteen fractions of 1.0 ml followed by 145 fractions of 0.3 ml were collected in scintillation vials. The radioactivity in each fraction was determined by liquid scintillation counting using ACS. An aliquot of [3H]-(+)-anti-BaPDE:dGuo, prepared as described previously (21), was added to each sample prior to HPLC analysis.

Individual BaP:deoxyribonucleoside adducts for acid hydrolysis studies were prepared by immobilized boronate chromatography and reverse-phase HPLC without the addition of the [3H]-(+)-anti-BaPDE:dGuo marker. A 10-µl aliquot of each HPLC fraction was analyzed for radioactivity by liquid scintillation counting using ACS, and the fractions containing each peak of radioactivity were pooled.

Hydrolysis of BaP:Deoxyribonucleoside Adducts. The BaP:deoxyribonucleoside adducts were hydrolyzed to BaP:purine adducts as described previously (23), using a modification of the procedure described by Osborne et al. (28). A portion of the pooled HPLC fractions containing a BaP:deoxyribonucleoside peak was evaporated under a N₂ stream to 5 µl. An aliquot of [3H]-(+)-anti-BaPDE:dGuo and 50 µl 0.1 N HCl were added. The sample was incubated for 24 h at 37°C. After neutralization, the hydrolys products were chromatographed by reverse-phase HPLC as described above.

BaP:deoxyribonucleosides were hydrolyzed to tetaols as described previously (23), using a modification of the procedure described by Shugart et al. (29). A portion of the pooled HPLC fractions containing a BaP:deoxyribonucleoside adduct peak was evaporated under a N₂ stream to 5 µl. For BaP:deoxyribonucleoside adduct MS₂, 50 µl 0.1 N HCl were added and the sample was incubated for 6 h at 80°C. For BaP:deoxyribonucleoside adducts MS₂ and MS₁, 50 µl 0.5 N HCl were added and the samples were incubated for 2 h at 80°C. After neutralization, the samples were chromatographed by reverse-phase HPLC with a mixture of the four benzo(a)pyrene tetaols purchased from the Chemical Repository, Division of Cancer Etiology, National Cancer Institute.

RESULTS

BaP:DNA Adducts in Normal Mammary Epithelial Cell Cultures. Normal human mammary epithelial cell cultures were exposed to [3H]-BaP (4 µM; specific activity, 6 Ci/mmoll) for 24 h. The DNA was isolated and the level of BaP bound was calculated to be 81 pmol/mg DNA. Analysis of the BaP:deoxyribonucleoside adducts by immobilized boronate chromatography demonstrated that 24% of the radioactivity eluted in the morpholine buffer (BaP:deoxyribonucleoside adducts not containing cis-cisoidal hydroxyl groups), and 76% eluted in the morpholine:sorbitol buffer (BaP:deoxyribonucleoside adducts containing cis-cisoidal hydroxyl groups). Reverse-phase HPLC analysis of the BaP:deoxyribonucleosides present in the morpholine buffer demonstrated one adduct peak, M2, that eluted in the same position relative to the...
[14C]-(+)-anti-BaPDE:dGuo marker as a syn-BaPDE:dGuo marker (Fig. 1A). Two BaP:deoxyribonucleoside adduct peaks were present in the reverse-phase HPLC profile of the morpholine:sorbitol buffer fractions (Fig. 1B; MS1 and MS2). The largest of which, MS2 (93% radioactivity), coeluted with the [14C]-(+)-anti-BaPDE:dGuo marker, and the other peak, MS1 (7% radioactivity), eluted in the same relative position as a (−)-anti-BaPDE:dGuo adduct marker. In these experiments, as well as in experiments with T47D cell cultures, a peak of unidentified radioactivity eluted in the breakthrough volume of the column.

**BaP:DNA Adducts in T47D Cell Cultures.** T47D cell cultures were exposed to [G-3H]BaP (4 µM; specific activity, 6 Ci/mmol) for 24 and 48 h. The DNA was isolated and the level of BaP bound was calculated to be 182 pmol/mg DNA after 24 h of exposure and 327 pmol/mg DNA after 48 h. The results are typical of values obtained in duplicate experiments. Analysis of the BaP:deoxyribonucleoside adducts by immobilized boronate chromatography demonstrated that after 24 h of exposure 19% of the radioactivity eluted in the morpholine buffer and 81% eluted in the morpholine:sorbitol buffer, whereas after 48 h of exposure, 46% of the radioactivity eluted in the morpholine buffer and 54% eluted in the morpholine:sorbitol buffer.

After 24 h of exposure the morpholine buffer fractions contained three adduct peaks that were resolved by reverse-phase HPLC (Fig. 2A; M0, M1, and M2). Peak M2 eluted in the same position relative to the [14C]-(+)-anti-BaPDE:dGuo marker as a syn-BaPDE:dGuo marker. Peak M1 eluted in the same relative position as both syn-BaPDE:dGuo and syn-BaPDE:dCyd markers. Peak M0 eluted prior to these adducts. Reverse-phase HPLC analysis of the morpholine:sorbitol buffer fractions demonstrated the presence of two adduct peaks (Fig. 2B; MS1 and MS2). The largest of these, peak MS2, coeluted with the [14C]-(+)-anti-BaPDE:dGuo marker. Peak MS1 eluted in the same relative position as a (−)-anti-BaPDE:dGuo adduct marker.

After 48 h of exposure the morpholine buffer fractions contained three adduct peaks that were resolved by reverse-phase HPLC (Fig. 2C; M0, M1, and M2). Peak M2 eluted in the same relative position to the [14C]-(+)-anti-BaPDE:dGuo marker as a syn-BaPDE:dGuo marker, peak M1 eluted in the same relative position as both syn-BaPDE:dGuo and syn-BaPDE:dCyd markers, and peak M0 eluted prior to these adducts. Reverse-phase HPLC analysis of the morpholine:sorbitol buffer fractions resolved three adduct peaks (Fig. 2D; MS0, MS1, and MS2). Peak MS2 coeluted with the [14C]-(+)-anti-BaPDE:dGuo marker, peak MS1 eluted in the same relative position as (−)-anti-BaPDE:dGuo, and MS0 eluted prior to these adducts.

**Hydrolysis of MS1.** To determine if MS1, the BaP:deoxyribonucleoside adduct that eluted in the same relative position as (−)-anti-BaPDE:dGuo in the HPLC profile of the morpholine:sorbitol buffer fractions obtained by immobilized boronate chromatography of adducts from both human mammary epithelial cells and human mammary carcinoma T47D cell cultures, was formed by reaction of (−)-anti-BaPDE with deoxyguanosine, a mixture of MS1, isolated from T47D cell cultures that had been exposed to [3H]BaP (4 µM; specific activity, 6 Ci/mmol) for 24 h, and [14C]-(+)-anti-BaPDE:dGuo was treated with 0.1 N HCl for 24 h at 37°C. Upon neutralization and reverse-phase HPLC analysis, one tritium and carbon-14 hydrolysis product was seen that eluted in fractions 100 to 115 (37% radioactivity tritium, 91% radioactivity carbon-14) (Fig. 3A); the elution position suggested that this adduct resulted from glycosidic bond cleavage to yield an anti-BaPDE:guanine adduct. Due to an isotope effect, the C-14 hydrolysis peak (fractions 105 to 115) eluted slightly later than the tritium peak. The early-eluting carbon-14 peak (fractions 45 to 50; 5% radioactivity C-14) coeluted with a UV marker of 7,10,8,9-tetraol. One additional peak of tritium (fractions
Fig. 3. Reverse-phase HPLC analysis of the 37°C acid hydrolysis products obtained from BaP:deoxyribonucleoside adducts MS1 and MS2. MS1 and MS2 were isolated from the DNA of human mammary carcinoma T47D cells after exposure to 4 μM [3H]BaP (6 Ci/mmol) for 24 h as described in "Materials and Methods." MS1 and MS2 were each mixed with an aliquot of [14C](+)-anti-BaPDE:dGuo and treated with 0.1 N HCl for 24 h at 37°C. After neutralization, the hydrolysis products were chromatographed by reverse-phase HPLC (MS1 (A); MS2 (B)). —, elution position of the hydrolysis products of [3H]MS1 or [14C]MS2; —, elution position of hydrolyzed [14C](+)-anti-BaPDE:dGuo.

45 to 61; 50% radioactivity) and one of C-14 (fractions 85 to 92; 3% radioactivity) were present: their elution positions corresponded to those of unhydrolyzed MS1 and unhydrolyzed [14C](+)-anti-BaPDE:dGuo, respectively. The finding that MS1 was formed by reaction of (+)-anti-BaPDE with deoxyguanosine was also demonstrated by hydrolysis of MS1 to tetratos. The optimum conditions for tetrool formation from MS1 were exposure to 0.5 N HCl for 2 h at 80°C. Reverse-phase HPLC analysis of the hydrolysis products with known (+)-syn-BaPDE:tetraols and (+)-anti-BaPDE:tetraols demonstrated that MS1 formed anti-BaPDE:tetraols upon acid hydrolysis (Fig. 4A). Four tritium peaks were seen: one coeluted with 7,10/8,9:tetraol (a tetrool formed from anti-BaPDE) with a maximum at fraction 44, and one coeluted with 7/8,9,10-tetraol (a tetrool formed from anti-BaPDE) with a maximum at fraction 71. A later-eluting tritium peak was also present with a maximum at fraction 103; this presumably resulted from the loss of deoxyribose from MS1 to give the anti-BaPDE:guanine adduct described in Fig. 3A. An additional tritium peak was present with a maximum at fraction 57 corresponding to unhydrolyzed BaP:deoxyribonucleoside adduct MS1. Thus, MS1 is formed from the (+) enantiomer of anti-BaPDE.

Hydrolysis of MS2. To determine if MS2, the BaP:deoxyribonucleoside adduct that coeluted with the [14C](+)-anti-BaPDE:dGuo marker in the HPLC profile of the morpholine:sorbitol buffer fractions obtained by immobilized boronate chromatography of adducts from both human mammary epithelial cells and human mammary carcinoma T47D cell cultures, was (+)-anti-BaPDE:dGuo, a mixture of 3H-MS2, isolated from T47D cell cultures that had been exposed to [3H]-BaP (4 μM; specific activity, 6 Ci/mmol) for 24 h, and [14C](+)-anti-BaPDE:dGuo was treated with 0.1 N HCl for 24 h at 37°C. Upon neutralization, reverse-phase HPLC analysis of the hydrolysis products demonstrated that both the tritium and carbon-14 hydrolysis products coeluted (Fig. 3B). Three tritium and three carbon-14 hydrolysis products were seen. The early-eluting peak (fractions 40 to 47; 14% radioactivity tritium, 28% radioactivity C-14) coeluted with the UV 7,10/8,9-tetraol marker. The late-eluting peak (fractions 93 to 107; 40% radioactivity tritium, 70% radioactivity C-14) corresponded to hydrocarbon:glycosidic bond cleavage yielding an anti-BaPDE:guanine hydrolysis product. Additional peaks of both tritium (fractions 67 to 76; 18% radioactivity) and C-14 (fractions 82 to 84; 2% radioactivity) were detected: these corresponded to unhydrolyzed 3H-MS2 and unhydrolyzed [14C](+)-anti-BaPDE:dGuo, respectively. The finding that MS2 was formed by reaction of (+)-anti-BaPDE with deoxyguanosine was also demonstrated by hydrolysis of MS2 to tetratos by exposure to 0.1 N HCl for 6 h at 80°C. Reverse-phase HPLC analysis of the hydrolysis products with known (+)-syn-BaPDE:tetraols and (+)-anti-BaPDE:tetraols demonstrated that MS2 formed anti-
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that MS2 formed anti-BaPDE:tetraols upon acid hydrolysis (Fig. 4B). Three tritium peaks were seen: the major peak coeluted with the UV 7,10,8,9-tetraol marker with a maximum at fraction 44, the tritium peak with a maximum at fraction 74 coeluted with the UV 7,8,9,10-tetraol marker, and the late-eluting peak (fractions 92 to 107) corresponded to hydroxycarbons:glycosidic bond cleavage yielding an anti-BaPDE:guanine adduct. Thus, MS2 is formed from (+)-anti-BaPDE.

Hydrolysis of M2. To determine if M2 was formed by reaction of (±)-syn-BaPDE with DNA, 3H-M2, isolated from T47D cell cultures that had been exposed to [3H]BaP (4 μM; specific activity, 6 Ci/mmol) for 24 h, was hydrolyzed to tetraols by exposure to 0.5 N HCl for 2 h at 80°C. Reverse-phase HPLC analysis of the hydrolysis products with known (±)-syn-BaPDE:tetraols and (±)-anti-BaPDE:tetraols demonstrated that M2 formed (±)-syn-BaPDE:tetraols upon acid hydrolysis (data not shown). Two tritium peaks were seen: one peak (32% radioactivity) coeluted with the UV 7,9,10,8-tetraol marker with a maximum in fraction 105, and the second peak of unidentified material (18% radioactivity) eluted with a maximum in fraction 49. As in all acid hydrolysis experiments, some radioactivity (50%) eluted in the breakthrough volume of the HPLC column (fractions 2 to 10). Thus, M2 is formed by reaction of (±)-syn-BaPDE with DNA.

DISCUSSION

The BaP:deoxyribonucleoside adducts formed in both human mammary epithelial cell cultures and the human mammary carcinoma T47D cell line were analyzed using immobilized boronate chromatography, reverse-phase HPLC, and acid hydrolysis techniques. The major BaP:deoxyribonucleoside adduct, MS2, was (+)-anti-BaPDE:dGuo. This finding confirmed the results of Macnicoll et al. (6), Stampfer et al. (8), and Bartley et al. (9) that suggested, based upon HPLC of the major BaP:deoxyribonucleoside adduct from cells with a marker of (+)-anti-BaPDE:dGuo, that (+)-anti-BaPDE:dGuo was formed in both human mammary epithelial and fibroblastic cells. MS1, which represented approximately 5% of the total BaP:deoxyribonucleoside adducts, resulted from reaction of the (-) enantiomer of anti-BaPDE with deoxyguanosine. This study using hydrolysis of MS1 to a BaP:guanine adduct and to BaP:7,8,9,10-tetraols provided the first evidence, other than chromatography, that (-)-anti-BaPDE was formed in cell systems and reacted with DNA in cells to form (-)-anti-BaPDE:dGuo. The third identifiable adduct, M2, was formed by reaction of (±)-syn-BaPDE with DNA.

Comparison of BaP:DNA adduct formation in human mammary epithelial cells and human mammary carcinoma T47D cells demonstrated that T47D cells have a high capacity for adduct formation (81 pmol/mg DNA in human mammary epithelial cells versus 182 and 327 pmol/mg DNA in human mammary carcinoma T47D cells exposed to 4 μM BaP for 24 and 48 h, respectively). Phillips et al. (24) found a binding level of only 11 pmol/mg DNA in rat mammary epithelial cells exposed to 15 μM BaP. The level of BaP bound to DNA in explant cultures from various human tissues exposed to 1.5 μM BaP for 24 h ranged from 0.1 to 3.2 pmol BaP/mg DNA (30–32). Early passage rodent embryo cell cultures exposed to 2 μM BaP contained 10 to 66 pmol BaP bound/mg DNA (22). Although variations in the culture and treatment conditions preclude direct comparison of these values with those obtained in the human mammary cell cultures, it is evident that both the human mammary epithelial cells and human mammary carcinoma T47D cells bind high levels of BaP to DNA. Similar BaP:deoxyribonucleoside adduct profiles were obtained in both cell types. The major BaP:deoxyribonucleoside adduct present in the HPLC profile of the morpholine buffer fractions from the immobilized boronate column was M2, a syn-BaPDE:dGuo adduct. The major BaP:deoxyribonucleoside adducts present in the HPLC profile of the morpholine:sorbitol buffer fractions were MS1 and MS2, (−)-anti-BaPDE:dGuo and (+)-anti-BaPDE:dGuo, respectively. The amounts of these were (pmol adduct/mg DNA): normal human mammary epithelial cells M2, 7.7; MS1, 3.9; MS2, 55.4; T47D cells (24 h) M2, 14.8; MS1, 8.7; MS2, 137.2; T47D cells (48 h) M2, 14.4; MS1, 21.1; MS2, 106.6. The relative proportion of these three BaP:deoxyribonucleoside adducts was similar in both normal human mammary epithelial cells and human mammary carcinoma T47D cells. MS2 was present in the largest amount in both cell systems and at both times of exposure. The ratio of M2:MS1 was similar in normal mammary cells and T47D cells exposed to BaP for 24 h. After 48 h of exposure to BaP, MS1 was formed to a greater extent than was M2 in the human mammary carcinoma T47D cell cultures.

The major pathways of formation of the DNA binding metabolites of BaP in human mammary cells are shown in Fig. 5. (+)-anti-BaPDE was responsible for greater than 75% of the BaP:deoxyribonucleoside adducts formed, (−)-anti-BaPDE for approximately 5%, and (±)-syn-BaPDE for about 9%. No other pathways of BaP metabolic activation to DNA-binding metabolites were detected in human mammary cell cultures. This is in contrast to BaP activation in Wistar rat cell systems. We have shown that of the three BaP:deoxyribonucleoside adducts that contained cis-vicinal hydroxyl groups formed in Wistar rat embryo cells, only one was formed from anti-BaPDE and (−)-anti-BaPDE:dGuo was not present (23). Phillips et al. (24) have shown that of seven BaP:deoxyribonucleoside adducts detected in rat mammary epithelial cells none were formed from (±)-anti-BaPDE. Thus, both rat mammary cell cultures and rat fibroblastic cultures differ from human mammary cell cultures in their pathways of metabolic activation of BaP to DNA-binding derivatives.

The BaP:deoxyribonucleoside adducts in the T47D mammary carcinoma cell line are similar to those in the human mammary epithelial cell cultures. The HPLC profile of these adducts is also similar to that observed by others in explant cultures from

Fig. 5. Schematic representation of the major pathways of formation of the DNA-binding metabolites of BaP in human mammary cells.
a number of human tissues such as colon, bronchus, and esophagus (19, 30—34) and in various tissues in mice and rabbits (35—38). This suggests that the T47D human mammary carcinoma cell line may prove a valuable model for studies of polycyclic aromatic hydrocarbon activation in human mammary tissue and other human tissues.

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