Patterns of DNA Adduct Formation in Liver and Mammary Epithelial Cells of Rats Treated with 7,12-Dimethylbenz(a)anthracene, and Selective Effects of Chemopreventive Agents

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INTRODUCTION

7,12-Dimethylbenz(a)anthracene (DMBA) is a prototype carcinogen that induces a high yield of mammary tumors in rats after a single feeding. We investigated the induction and chemoprevention of DNA adducts in female Sprague Dawley rats receiving DMBA by gavage according to a variety of treatment schedules. The patterns of 32P-postlabeled DNA adducts in liver and mammary epithelial cells were similar to those produced by the in vitro reaction of metabolically activated DMBA with calf thymus DNA. There was a high and statistically significant correlation between dose of DMBA administered to rats (0, 0.6, 2.4, and 12 mg/kg body weight) and levels of DNA adducts in both types of cells. The regression lines relating DMBA doses to total DNA adduct levels were significantly divergent and crossed at 1.5 mg/kg body weight, indicating that, at lower doses, the formation of DNA adducts is more intense in target mammary cells, whereas at higher doses, DNA adduct levels are more elevated in liver cells, presumably due to the greater metabolic capacity of this organ. When the rats were sacrificed 7 days rather than 2 days after DMBA administration, DNA adduct levels were approximately halved in both liver and mammary cells. The observed patterns can be interpreted based on toxicokinetic factors, local and distant metabolism, removal of DNA adducts by excision repair, and cell proliferation rate. Of three chemopreventive agents given with the diet to rats treated with 12 mg of DMBA, 5,6-benzoflavone (1650 ppm) was the most effective, inhibiting DNA adduct formation in liver and mammary cells by 96.5 and 83.5%, respectively. Feeding of 1,2-dithiole-3-thione (600 ppm) inhibited this biomarker by 68.5 and 50.2%, whereas butyl hydroxyanisole (BHA; 5000 ppm) showed no significant inhibition in the liver (46.5%) but was ineffective in mammary cells (29.0%, not significant). These data correlate nicely with the results of a parallel study in which 5,6-BF appears to be more effective than the broad-spectrum inducer 5,6-BF, which, like other dithiolethiones, was shown to possess antigenotoxic activity and anticarcinogenic properties in experimental test systems (13). The other two chemicals are monofunctional agents, which primarily induce phase II enzymes. They are the phenol BHA, a synthetic antioxidant (14) used as a food preservative, and 1,2-D3T, which, like other dithiolethiones, was shown to possess antigenotoxic and anticarcinogenic properties in experimental test systems (15). A further goal was to evaluate the relationships between inhibition by chemopreventive agents of DNA adduct formation in liver and mammary cells, the hemoglobin adducts of DMBA metabolites tested in the same animals, and the mammary tumor yield, which, along with the modulation of metabolic factors, was investigated in parallel groups of rats treated with DMBA and chemopreventive agents according to comparable treatment schedules.

MATERIALS AND METHODS

Reagents for in Vitro Experiments. DMBA was purchased from Fluka (Buchs, Switzerland), and calf thymus DNA was purchased from Sigma

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3 The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; PAH, polycyclic aromatic hydrocarbon; 5,6-BF, 5,6-benzoflavone; BHA, butylated hydroxyanisole; 1,2-D3T, 1,2-dithiole-3-thione; GSH, glutathione.

4 L. L. Song et al., manuscript in preparation.
Chemical Co. (St. Louis, MO). S9 mix is a NADPH-generating mixture containing 10% liver S12 fractions from Arocot 1254-pretreated Sprague Dawley rats (16).

**Animals.** Female Sprague Dawley rats were used throughout the study. Twelve 12-week-old rats (Morini, S. Polo d’Enza, Italy) were used in a preliminary experiment carried out at the University of Genoa. Forty-five 4-week-old rats (virus-free colony no. 218; Harlan Sprague Dawley, Inc., Indianapolis, IN) were received at the University of Alabama at Birmingham to the University of Genoa, where they arrived still frozen, and maintained at 80°C until use. The treatment groups were blind-coded, and the values recorded in rats receiving the same dose of DMBA, in the absence of chemopreventive agents.

**DNA Extraction.** Aliquots of liver (100 mg) and mammary cells (50 mg) were thawed and homogenized in a Potter-Elvehjem apparatus at 4°C in 250 mm sucrose, 5 mm 1,4-DTT, and 50 mm Tris-HCl (pH 7.6). DNA was isolated by solvent extraction using an automatic extractor (Genepure 341; Applied Biosystems, Foster City, CA) according to the method of Gupta (19), with some modifications, as described previously (20). Homogenized cells were sequentially treated first with a mixture of RNase A and T1 for 1 h and then with proteinase K for 5 h at 55°C, followed by two extractions with a phenol-water-choroform mixture and two extractions with chloroform only. To increase DNA recovery from mammary epithelial cells, we added 30 μl of a silica-based DNA binding reagent (Quik-Precip; Edge-Biosystems, Gaithersburg, MD) during alcoholic precipitation. The whole procedure was performed in a helium atmosphere by using phenol of the highest available quality to avoid induction of oxidative DNA damage during extraction. The extracted DNA was analyzed and checked for purity by spectrophotometric analysis. The following absorbance ratios were obtained: $A_{260nm}$/$A_{230nm}$ $\geq$ 1.5; $A_{260nm}$/$A_{280nm}$ $\geq$ 1.8; and $A_{260nm}$/$A_{230nm}$ $\geq$ 1.70 and $\leq$ 1.95. The amounts of DNA obtained from 1 mg of wet tissue were, on average, 1.87 and 0.27 μg for liver and mammary cells, respectively.

**Detection of DNA Adducts by 32P-Postlabeling.** Postlabeling procedures were basically performed as reported by Gupta (21), with some modifications. Six μg of DNA were hydrolyzed to deoxyribonucleoside 3'-monophosphate by micrococcal nuclease (0.04 units/μg DNA) and spleen phosphodiesterase (1 milliunit/μg DNA) in 20 mM sodium succinate-10 mM calcium chloride (pH 6.0; 37°C for 3.5 h). Normal nucleotides were digested by adding 5 μl of nuclease P1 (2.77 units) in 3 mM zinc chloride-250 mM sodium acetate (pH 5.0; 37°C for 40 min). Polynucleotide kinase (8 units) in 6 μl of 200 mM bicine, 100 mM magnesium chloride, 100 mM 1,4-DTT, and 10 mM spermidine (pH 9.5) was added. The 32P-labeling reaction was performed by including 4 μl of [γ-32P]ATP (16 μCi/μl, specific activity, $\geq$ 6,000 Ci/mmol; ICN Biomedicals, Irvine, CA) in the mix and incubating at 22°C for 40 min. Labeled samples were developed on either 10 × 10 cm (preliminary in vivo experiment) or 12.5 × 10 cm (preliminary in vitro experiment and final in vivo experiment) polyethyleneimine-coated cellulose sheets (Macherey & Nagel, Düren, Germany) according to a multidirectional TLC system. The first development (D1; downward direction in Fig. 1) was performed in 1 m sodium phosphate (pH 6.0) overnight by stapling a wick paper at the top edge of the sheet. The second development (D2) was performed in 3 mM lithium formate-8.5 mM urea (pH 3.8) at 180°C, as compared with D1 (from bottom to top in Fig. 1). A third development (D3) was performed in 0.6 mM lithium chloride-8.5 mM urea (pH 8.0) at 90°C clockwise, as compared with D2 (from left to right in Fig. 1). The final development was in 1.7 m sodium phosphate in the same direction as the final development of the preliminary experiment carried out at the University of Genoa.
RESULTS

Preliminary Experiments. Two preliminary experiments were carried out. The first was an in vitro experiment in which we mixed either 1 mM DMBA or its solvent (DMSO), 100 μl of S9 mix, and 300 μg of calf thymus DNA in 1.3 ml of PBS (pH 7.4). After contact for 20 min at 37°C, DNA was purified, and DNA adducts were measured. No adduct was detected in DMBA-free calf thymus DNA, at an assumed sensitivity threshold of 0.1 adducts/10^8 nucleotides. Conversely, five well-distinguished autoradiographic spots, the patterns of which were comparable with those shown in Fig. 1, were detected in DMBA-treated DNA. The levels (means ± SD of triplicate determinations) were 198 ± 43, 110 ± 51, 10.1 ± 3.4, 5.5 ± 2.8, and 2.4 ± 1.8 adducts/10^8 nucleotides for the five individual spots and 326 ± 48 adducts/10^8 nucleotides for total DNA adducts.

The other preliminary experiment aimed at comparing two methods for isolating mammary epithelial cells. For this purpose, 12 female Sprague Dawley rats were treated by gavage with DMBA in corn oil (25 mg/kg body weight). After 48 h, the animals were sacrificed, and tissue fragments containing mammary glands were frozen in liquid nitrogen and then stored at -80°C. The thawed specimens were divided into two groups and comparatively processed by following the protocols described by Moon et al. (17) and Moore et al. (18) for isolating mammary epithelial cells. On average, the two methods yielded 81 and 28 mg of epithelial cells, respectively, from 1 g of s.c. tissue containing mammary glands. 32P-postlabeling of the DNA extracted from the mammary epithelial cells isolated by the two methods yielded a total of 35.1 ± 12.9 and 37.9 ± 10.3 adducts/10^8 nucleotides, respectively (means ± SD of three samples each).

General Patterns of 32P-Postlabeled DNA Adducts. Table 1 provides an outline of the results obtained in the nine experimental groups. The results are means ± SD of DNA adduct levels measured in five rats per group, each generated in two separate experiments (mammary epithelial cells) or three separate experiments (liver cells). Therefore, Table 1 synthesizes the results of 90 32P-postlabeling analyses with mammary epithelial cells and of 135 analyses with liver samples.

With an assumed sensitivity threshold of 0.1 adducts/10^8 nucleotides, no DNA adduct was detected in either tissue of untreated rats (controls). Conversely, DNA adducts were formed in both liver and mammary cells of all eight groups of rats treated with DMBA, with varying intensity depending on the experimental group. As shown in Fig. 1, the general patterns showed the consistent presence of one major adduct (spot 1) and four minor adducts (spots 2–5) in both liver and mammary cells. The size of the spots reproduced in Fig. 1 indicates, on an arbitrary scale, the levels of DNA adducts corresponding to either 1 mg or 10 mg of DMBA/kg body weight, as calculated from dose-response regression lines (see below).

Dose-Effect Relationships in the in Vivo Formation of DMBA-Induced DNA Adducts. Dose-effect relationships were evaluated by relating the dose of DMBA administered to rats (single dose of 0.6, 1.2, 6.0, and 12 mg/kg body weight) and DNA adduct levels in liver and mammary epithelial cells of Sprague-Dawley rats sacrificed 2 days after DMBA administration.

![Fig. 1. Schematic representation of the autoradiographic patterns of 32P-postlabeled DNA adducts formed in liver and mammary epithelial cells of Sprague Dawley rats treated by gavage with a single administration of DMBA. The surface areas of each one of the five spots reproduces, on a relative arbitrary scale, the levels of DNA adducts/10^8 nucleotides formed after a hypothetical administration of either 10 mg/kg body weight (light gray outer circles) or 1 mg/kg body weight (dark gray inner circles), as calculated from the regression lines relating the DMBA dose to DNA adduct levels (see Table 2).](image)

![Liver cells](image)

![Mammary epithelial cells](image)

Table 2. Correlation between dose of DMBA (0, 0.6, 2.4, or 12 mg/kg body weight) and DNA adduct levels in liver and mammary epithelial cells of Sprague-Dawley rats sacrificed 2 days after DMBA administration

<table>
<thead>
<tr>
<th>Organ or cells</th>
<th>Autoradiographic spot</th>
<th>r</th>
<th>P</th>
<th>Regression line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>1</td>
<td>0.995</td>
<td>&lt;0.05</td>
<td>y = 0.587 + 1.817x</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.997</td>
<td>&lt;0.01</td>
<td>y = 0.079 + 0.7x</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.989</td>
<td>&lt;0.05</td>
<td>y = -0.335 + 0.462x</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.992</td>
<td>&lt;0.01</td>
<td>y = -0.105 + 0.222x</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.986</td>
<td>&lt;0.05</td>
<td>y = -0.312 + 0.385x</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.998</td>
<td>&lt;0.01</td>
<td>y = -0.127 + 3.588x</td>
</tr>
<tr>
<td>Mammary epithelial cells</td>
<td>1</td>
<td>0.987</td>
<td>&lt;0.05</td>
<td>y = 0.695 + 1.522x</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.932</td>
<td>0.07</td>
<td>y = 0.435 + 0.293x</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.972</td>
<td>&lt;0.05</td>
<td>y = 0.155 + 0.193x</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.962</td>
<td>&lt;0.05</td>
<td>y = 0.187 + 0.131x</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.974</td>
<td>&lt;0.05</td>
<td>y = 0.182 + 0.259x</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>0.978</td>
<td>&lt;0.05</td>
<td>y = 1.617 + 2.403x</td>
</tr>
</tbody>
</table>

a x is the dose of administered DMBA (mg/kg body weight), and y is the level of DNA adducts/10^8 nucleotides.
2.4, or 12 mg/kg body weight plus 0 for controls) to the levels of individual spots and total DNA adducts measured in liver and mammary cells of rats sacrificed 2 days after DMBA administration in the absence of chemopreventive agents. As shown in Table 2, the dose-effect correlation coefficients (r) were very high, all of them being statistically significant (r was of borderline significance only in the case of spot 2 in mammary cells). Therefore, the regression lines reported in Table 2 could reliably be used for further analyses. The surface area of each of the five spots reported in Fig. 1 reproduces, on a relative scale, DNA adduct levels formed after hypothetical administrations of DMBA at either 10 mg or 1 mg/kg body weight, as calculated from the regression lines. As shown in Fig. 2, with an increase in DMBA dose, the regression line for liver cells is steeper than that for mammary cells. This differential trend, evaluated by parallel test of the regression lines, is statistically significant (P < 0.05). In other words, this sort of calibration curve indicates that the formation of DNA adducts is higher in mammary epithelial cells at DMBA doses that are <1.5 mg/kg body weight, whereas at DMBA doses exceeding that level, the formation of DNA adducts tends to be increasingly higher in liver cells.

Persistence of DMBA-induced DNA Adducts. The persistence of DNA adducts was evaluated in groups of rats receiving a single dose of DMBA, either at 12 mg or 0.6 mg/kg body weight, and sacrificed either 2 or 7 days after DMBA administration (Table 1). Compared with the rats sacrificed 2 days after the DMBA treatment, total DNA adduct levels in liver and mammary cells were 56.0 and 46.8%, respectively, in the animals sacrificed 7 days after administration of the 12-mg dose, and 30.3 and 58.3%, respectively, after administration of the 0.6 mg dose. Although remarkable in terms of percentage decrease, the differences recorded at the 0.6 mg dose were not statistically significant. Those recorded at the 12-mg dose were significant for spots 2 (P < 0.01), 3 (P < 0.05), 4 (P < 0.01), and 5 (P < 0.05) and total DNA adducts (P < 0.01) in liver cells and for spots 1 (P < 0.01) and 3 (P < 0.05) and total DNA adducts (P < 0.05) in mammary epithelial cells.

Effects of Chemopreventive Agents. Of the three chemopreventive agents under study, 5,6-BF was the most effective inhibitor of DNA adduct formation, decreasing total DNA adducts in liver and mammary cells by as much as 96.5% and 83.5%, respectively. As shown in Table 1, not only total DNA adducts but also all five individual spots were significantly decreased in both liver and mammary cells, as compared with rats receiving 12 mg DMBA, in the absence of chemopreventive agents.

BHA was the less effective inhibitor, decreasing total DNA adducts in liver and mammary cells by 46.3 and 29.0%, respectively. Inhibition was significant for spots 1, 2, and 4 and for total DNA adducts in the liver, whereas it did not reach the statistical significance threshold for DNA adducts in mammary cells.

1,2-D3T had an intermediate efficacy, decreasing total DNA adducts in liver and mammary cells by 68.5 and 50.2%, respectively. The decrease was significant for all spots and total DNA adducts in the liver and for spot 1 and total DNA adducts in mammary cells (Table 1).

DISCUSSION

The analysis of 32P-postlabeled adducts resulted in the consistent detection of five autoradiographic spots in calf thymus DNA, treated in vitro with DMBA in the presence of an exogenous metabolic system, as well as in liver and mammary epithelial cell DNA of rats receiving DMBA by gavage. As inferred from literature data (24, 25), these spots are likely to reflect reactions between anti-DMBA-dihydriodiolepoxide and either dG (spots 1 and 5) or dA (spot 2) and between syn-DMBA-dihydriodiolepoxide and either dG (spot 3) or dA (spot 4). Because spot 1 accounted for more than half of the DNA adducts formed in both liver and mammary cells, anti-DMBA-dihydriodiolepoxide-dG appears to be the predominant DNA adduct.

A high correlation was found to occur between the dose of administered DMBA and the levels not only of total DNA adducts but also of all five individual spots in both liver and mammary epithelial cells. The regression lines relating the DMBA dose with DNA adduct levels in these two types of cell populations were significantly divergent and crossed at a dose of 1.5 mg/kg body weight DMBA. Thus, by reducing DMBA doses, DNA adduct levels appear to be higher in mammary cells, whereas by increasing DMBA doses, they become higher and higher in liver cells.

Table 3: Inhibition by dietary chemopreventive agents of the formation of DNA adducts and hemoglobin adducts in Sprague-Dawley rats treated with a single intragastric administration of DMBA (12 mg/kg body weight) and of tumor yield in rats of the same strain treated with a single intragastric administration of DMBA (15 mg/kg body weight)

<table>
<thead>
<tr>
<th>Chemopreventive agent (dietary dose)</th>
<th>DNA adducts&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Hemoglobin adducts&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mammary tumors&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6-BF (1650 ppm)</td>
<td>Liver: 96.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Mammary: 83.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Incidence: 82.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>1,2-D3T (600 ppm)</td>
<td>Liver: 68.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Mammary: 50.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Incidence: 47.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>BHA (5000 ppm)</td>
<td>Liver: 46.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Mammary: 29.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Multiplicity: 5.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> This study.
<sup>b</sup> L. L. Song et al., manuscript in preparation (see Footnote 4).
<sup>c</sup> Statistically significant inhibition.
that DMBA metabolites formed in the liver can travel to the mammary gland via the blood circulation, and in addition, DMBA itself can be metabolized in mammary cells (27, 28). Another tentative hypothesis is that differences in constitutive and inducible cytochrome P450 isoforms involved in DMBA metabolism may partially contribute to the organ-specific differences in DNA adduct dose responses observed.

Besides metabolic factors, other mechanisms can account for the selective formation of DNA adducts in different organs and cell types (29, 30). One is toxicokinetics and, in particular, the so-called first-pass effect, in which, after intragastric administration of DMBA, subsequent uptake via the portal system is much more pronounced in the liver than in the mammary gland. On the other hand, the greater first-pass effect and metabolizing capacity is compensated in the liver by a quite efficient removal of DNA adducts via excision repair (30). A further crucial mechanism is represented by the cell proliferation rate, which is higher in mammary epithelial cells than in hepatocytes. More intense cellular turnover tends to dilute DNA adducts accordingly to lower their persistence. At the same time, cell proliferation is an essential mechanism favoring all stages of the carcinogenesis process (30). Due to the balance between these contrasting mechanisms, the persistence of DNA adducts was similar in liver and mammary cells, their levels being approximately halved when measured 7 rather than 2 days after DMBA administration.

The observed linearity of the dose-response curves relating the doses of administered DMBA to the levels of DNA adducts provides an ideal technical premise for the assessment of protective effects. The three investigated chemopreventive agents, all of them given with the diet, displayed a differential ability to inhibit the formation of DMBA-induced DNA adducts. In fact, the efficacy ranked as follows: 5,6-BF > 1,2-D3T > BHA. These data are in agreement with the evaluation of DMBA-hemoglobin adducts in the same animals, which showed inhibitions of 80.0% with 5,6-BF, 44.0% with 1,2-D3T, and 0% with BHA. Moreover, inhibition by these chemopreventive agents of DNA adducts induced by DMBA (12 mg/kg body weight) is well correlated with inhibition of the yield of mammary tumors, as assessed in a parallel study following a very similar treatment schedule, except that DMBA was given at a slightly higher dose (15 mg/kg body weight). In fact, 5,6-BF inhibited tumor incidence by 82.4% and multiplicity by 92.6%; 1,2-D3T inhibited tumor incidence by 47.1% and multiplicity by 80.0%; and BHA did not produce significant effects, decreasing tumor incidence by 5.9% and multiplicity by 7.4% only.

As summarized in Table 3, with the exception of a significant ability of BHA to inhibit DNA adducts in liver cells, which is well explained by distinctive metabolic effects of this phenolic antioxidant in hepatocytes and mammary cells (see below), there is a striking concordance between inhibition of early biomarkers, either in target cells (DNA adducts in mammary epithelial cells) or in the main metabolizing organ (DNA adducts in liver cells) or at a systemic level (hemoglobin adducts) and inhibition of mammary tumor incidence and multiplicity. These findings support the validity of early biomarkers that measure the ability of the biologically effective dose to predict the final pathological event in the DMBA rat mammary tumor model. Such a conclusion is in agreement with the general postulate that inhibition of DNA adducts is a reliable indicator of chemoprevention of tumors. In fact, in most cases, these promutagenic lesions are a necessary step, the down-regulation of which breaks the chain of events leading to the disease (31). A parallelism between inhibition of mammary DNA adducts and tumors in DMBA-treated rats was also observed after dietary administration of sodium selenite (7), 1,4-phenylenbis(methylene)selenocyanate (8), aliphatic selenocyanates (10), or butyl phthalate (6) or after i.p. injections of butylated hydroxytoluene (3), rosemary extract and its constituent carnosol (4), or curcumin (5).

Chemopreventive agents can inhibit genotoxicity and carcinogenicity through a large variety of mechanisms (32, 33). Because the agents under study had parallel effects on the formation of DNA adducts and on the yield of mammary tumors, it is likely that they worked at a level preceding binding of DMBA metabolites to DNA, presumably by altering the metabolism of this P4A. In any case, inhibition by these chemopreventive agents was of the same order of magnitude for the individual DNA adducts in both types of cells, which indicates that there was no preferential effect toward the two DMBA-dihydrodiol epoxide enantiomers.

All three chemopreventive agents under study are well-known phase II enzyme inducers and have additional effects on the metabolism of xenobiotics. For instance, 5,6-BF binds the Ah receptor and, accordingly, induces a variety of cytochrome P-450 isoforms, including CYP1A1, CYP1A2, and CYP1B1 (13). It was also proposed that 5,6-BF can inhibit DMBA mammary carcinogenesis by interfering with the oxidative metabolism of this carcinogen (34). Interestingly, irrespective of DMBA administration, 5,6-BF had striking effects on phase II activities in Sprague Dawley rats by inducing NADP(H) quinone reductase and GSH S-transferase and by increasing GSH levels in different tissues, including the mammary gland. BHA had no influence on these parameters in the mammary gland, whereas substantial induction of phase II enzymes and GSH content were seen in the liver. Similar conclusions were drawn in the same rat model after dietary administration of butylated hydroxytoluene (35). This differential metabolic effect nicely correlates with our finding that dietary BHA selectively inhibited the formation of DNA adducts in the liver but not in mammary epithelial cells and with the failure of this phenol to modulate the mammary carcinogenesis of DMBA.

There is no doubt that the selective induction of phase II activities by monofunctional inducers is an important mechanism of inhibition of genotoxicity and carcinogenicity (32, 33). However, as shown in this study by the potent inhibition produced by 5,6-BF, broad-spectrum inducers may even be more effective. Indeed, as also reported with other chemopreventive agents (33, 36), the ideal detoxification may be the one provided by agents that increase metabolism of the procarcinogen, while simultaneously inactivating ultimate carcinogen metabolites by increasing the concentration of nucleophiles and achieving detoxification and excretion via phase II pathways.

REFERENCES


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