Effects of Retinoids on Metabolizing Enzymes and on Binding of Benzo(a)pyrene to Rat Tissue DNA

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ABSTRACT

Retinoids have a prophylactic and, in some cases, a therapeutic effect on certain malignant epithelial lesions in animals. These compounds also exert a profound control on cellular differentiation and growth. Some effects of retinoids include a delay in the appearance of carcinogen-induced papillomas on the skin of mice (1), a reduction in the incidence of bladder carcinomas in rats (5-7), and the tissues were harvested 24 h later. Binding to lung DNA was reduced by 23 and 11%, respectively, in the 13cisRA- and 4HPR-dosed rats. No differences were observed in binding to kidney. Thus, retinoids, by altering the metabolism of carcinogens, could influence the initiation stage of carcinogenesis.

INTRODUCTION

Retinoids have a prophylactic and, in some cases, a therapeutic effect on certain malignant epithelial lesions in animals. These compounds also exert a profound control on cellular differentiation and growth. Some effects of retinoids include a delay in the appearance of carcinogen-induced papillomas on the skin of mice (1), a reduction in the incidence of bladder cancer in rats and mice (2-4), and a reduction in the incidence of mammary carcinomas in rats (5-7). Other tissues in which retinoids have been reported to exert a chemopreventive effect include trachea (8), liver (9), and stomach (10). Since, in most studies, retinoids are given to animals after administration of carcinogen, it is widely accepted that retinoids inhibit tumors by acting at the promotional stage of carcinogenesis (11).

There are a few reports, however, in which retinoids given prior to carcinogen caused an inhibition of tumor development (12-15). The carcinogens used in these studies required metabolic activation, suggesting that retinoids could alter the levels of metabolizing enzymes, and thereby affect the initiation phase of tumorigenesis. Leo et al. (16) reported that rats fed diets containing 100 times the normal amount of vitamin A had an increased level of cytochrome P-450, which was associated with an enhanced conversion of RA to polar metabolites. In addition, pretreatment of animals with p.o. doses of retinoids induced the metabolism of RA in the tissues of vitamin A-deficient hamsters (17, 18). A class of cytochrome P-450 mixed-function oxidases different from those induced by PB and MC was responsible for this increased metabolism. Retinoids also altered carcinogen-induced sister chromatid exchange and mutagenesis in vitro (19, 20).

We present evidence that retinoids may affect initiation by altering carcinogen metabolism. We compared retinoid-induced changes in hepatic QR, GST, and AHH with changes induced by BHT and Sudan III, compounds known to affect initiation, and determined the effects of these compounds on the in vivo binding of BaP to DNA.

MATERIALS AND METHODS

Materials. 13cisRA and 4HPR were prepared at Southern Research Institute by Y. F. Shealy and J. L. Frye under contract to the National Cancer Institute. RA was purchased from ICN Nutritional Biochemicals, Cleveland, OH. Retinoids, prepared in sesame oil and stored under nitrogen, were stable, as determined by high-performance liquid chromatography, for the length of the experiment. Sudan III, BHT, and all other reagents were purchased from Sigma Chemical Co., St. Louis, MO. [3H] BaP (88 Ci/mmol) was purchased from Amersham Corp., Arlington Heights, IL, and was purified further by thin-layer chromatography. [14C] BAPl7,8-diol was purchased from National Cancer Institute Chemical Carcinogen Reference Standard Repository, Midwest Research Institute, Kansas City, MO. 3-Hydroxy-BaP, used as a standard in the AHH assay, was supplied by Dr. Hans Falk, National Institute of Environmental Sciences, Research Triangle Park, NC.

Carcinogens. Male Sprague-Dawley rats (Charles River Laboratories, Portage, MI), weighing 140 ± 50 g (SD) were quarantined for 14 days prior to dosing and were housed 4/4 in suspended polycarbonate cages. An open formula NIH-07 diet and water were administered ad libitum. Animals were housed and dosed in American Association for Accreditation of Laboratory Animal Care-accredited facilities.

Animal Experiments. For the enzyme induction experiments, rats were dosed daily by gavage for 4 days with 13cisRA, RAc, 4HPR, BHT, or Sudan III at 235, 320, 800, 300, or 25 mg/kg/day, respectively. Control rats were dosed only with the vehicle, sesame oil. Animals were killed on the fifth day by collapse of the thoracic cavity while under ether anesthesia. Livers were removed and homogenized in 0.25 M sucrose (3.0 ml/g of tissue) at 4°C and centrifuged for 25 min at 9,000 X g. Portions of the cytosol fractions were centrifuged immediately for QR activity, and the remaining preparations were stored frozen at -20°C and assayed for GST at a later time. Liver microsomal pellets were washed in 8-10 ml of 10 mM Tris-HCl (pH 7.4) in 0.25 M sucrose, resuspended in 1 volume of this medium, and stored frozen at -20°C until assayed for AHH.

1 The abbreviations used are: RA, all-trans-retinoic acid; 13cisRA, 13-cis-retinoic acid; RAc, retinyl acetate; 4HPR, a-hydroxyphenyl)retinamide; QR, quinone reductase; GST, glutathione-S-transferase; AHH, arylhydrocarbon hydroxylase; BHT, butylated hydroxytoluene; BaP, benzo[a]pyrene; BaP-7,8-diol, (13,13-trans-7,8-dihydrobenzo(a)pyrene-7,8-diol; MC, 3-methylcholanthrene; PB, phenobarbital; SDS, sodium dodecyl sulfate; PCN, pregnenolone-16a-carbonitrile.
RETINOID MODIFICATION OF CARCINOGEN METABOLISM

13cisRA and BHT were chosen for additional time-course and dose-response experiments, in which rats received 13cisRA or BHT by gavage at 235 and 600 mg/kg/day for 1, 2, 4, 7, or 14 days. The livers were processed and enzyme levels were determined as described. In a separate experiment, rats received either 13cisRA at doses of 1, 15, 60, 120, 235, or 400 mg/kg/day or BHT at doses of 0.73, 44, or 172 mg/kg/day for 7 days. On the eighth day, the animals were killed and the levels of hepatic enzymes determined.

For BaP-binding experiments, animals were dosed for 7 days with 13cisRA, RAC, 4HPR, BHT, or Sudan III at 120, 80, 600, 300, or 25 mg/kg/day, respectively. The dose for the 13cisRA-dosed animals was selected as the amount that gave maximal induction of hepatic QR and near maximal suppression of AH. The other retinoids were given to the maximum nontoxic dose as determined in prior experiments. On the eighth day, animals received ip. injection of [14C]BaP (0.267 Ci/mmol) in corn oil at a dose of 2 mg/kg (2.1 nCi/kg). Animals were killed by collapse of the thoracic cavity 24 h after the injection of BaP, and the tissues were placed immediately on dry ice and stored at −80°C until processed.

Enzyme and Microsomal Assays. Cytosolic QR was determined by the method of Ernster (21) as modified and described in detail by Benson et al. (22). The assay, performed at 25°C, measured the initial velocity of the dicoumarol-sensitive reduction of dichloroindolphenol at 600 nm (ε = 2.1 × 10^4 M−1 cm−1). Cytosolic GST was determined by the method of Habig et al. (23) with 1-chloro-2,4-dinitrobenzene as a substrate. The assay, performed at 25°C, measured the appearance of product at 240 nm (ε = 9.6 M−1 cm−1). Microsomal AH activity was measured with a modified procedure of Bowden et al. (24). One AH unit was expressed as the amount of enzyme that catalyzes, in 30 min at 37°C, the formation of alkali-extractable BaP products producing a fluorescence equivalent to 1 pmol of synthetic 3-hydroxybenzo(a)pyrene. Protein was measured by the method originally described by Lowry (25). Cytosomes P-450 and b, levels, with respective extinction coefficients of 91 and 171 M−1 cm−1, were determined by the method of Omura and Sato (26). A one-tailed Student's t test was used to determine differences among the groups. Octylamine difference spectra were obtained according to the method of Jefcoate et al. (27).

In Vitro Binding of BaP Metabolites to Microsomal Proteins. In a NADPH-dependent system, we determined the extent of binding of BaP-7,8-diol metabolites to microsomal proteins from control, 13cisRA-, and BHT-dosed animals. These animals were dosed using the same protocol as the in vivo binding experiments, i.e., for 7 days with either sesame oil or 13cisRA and BHT at 120 and 300 mg/kg/day.

Cytosolic QR remained at control levels at all doses of 13cisRA. At the selected dose levels, both 13cisRA and BHT suppressed AHH, with 13cisRA showing a gradual return to control levels after 14 days (Fig. 1). BHT caused a rapid and greater increase, compared to 13cisRA, in the activity of this enzyme, with maximum induction occurring in 4 days. Over 14 days, 13cisRA had no effect on the levels of GST, but BHT caused a significant increase in the activity of this enzyme. Both 13cisRA and BHT caused a rapid reduction in AHH levels; but with BHT, there was a gradual return to control levels of this enzyme after 14 days.

The dose-response curve for the induction of QR by 13cisRA was unusual in that there was a small but significant and reproducible increase in enzyme activity at a dose of 1 mg/kg (Fig. 2). With intermediate doses, the activity was near control levels, but there was a second rise in activity at higher doses. GST remained at control levels at all doses of 13cisRA. At the selected dose levels, both 13cisRA and BHT suppressed AH, but this activity returned to control levels in animals given the highest dose of 13cisRA.

Cytochromes P-450s. Compared with controls, 13cisRA-dosed animals had slightly elevated levels of cytochrome P-450 and a CO-difference spectrum maximum that was shifted slightly towards 448 nm (Table 2). In addition, 13cisRA caused a shift in the octylamine difference spectrum to a type IIa or high-spin form (Fig. 3). The level of cytochrome P-450 was also elevated in the 4HPR-dosed animals but was unchanged in the RAc group (Table 2). Neither 4HPR nor RAc caused shifts in the absorbance maximum of their respective CO-difference spectra. Compared with controls, RAc but not 4HPR or 13cisRA caused lower levels of cytochrome b₂.

Among the retinoid-dosed animals, only minor changes were observed on SDS gels in the intensities of bands corresponding to the 9 purified cytochrome P-450 standards (Fig. 4), which cover a molecular weight range of 48,000–56,000. As evidenced
RETINOID MODIFICATION OF CARCINOGEN METABOLISM

Table 1 Liver enzyme profiles from male Sprague-Dawley rats dosed with retinoids

Animals were dosed for 4 days with 13cisRA, RAc, 4HPR, BHT, or Sudan III at 23.5, 32.0, 80.0, 60.0, or 25 mg/kg/day, respectively. Control animals received sesame oil. Values are means ± SD, 5 rats/group.

<table>
<thead>
<tr>
<th>Treatments*</th>
<th>Quinone reductase (nmol/min/mg)</th>
<th>Glutathione-S-transferase (nmol/min/mg)</th>
<th>Arylhydrocarbon hydroxylase (pmol/30 min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (sesame oil)</td>
<td>592 ± 203</td>
<td>920 ± 293</td>
<td>11,390 ± 4,648</td>
</tr>
<tr>
<td>13cisRA</td>
<td>956 ± 127*</td>
<td>1,145 ± 182</td>
<td>4,779 ± 1,452*</td>
</tr>
<tr>
<td>RAc</td>
<td>753 ± 379</td>
<td>805 ± 94</td>
<td>5,129 ± 2,139*</td>
</tr>
<tr>
<td>4HPR</td>
<td>710 ± 175</td>
<td>1,490 ± 190*</td>
<td>6,111 ± 3,102*</td>
</tr>
<tr>
<td>BHT</td>
<td>Not determined</td>
<td>1,450 ± 374*</td>
<td>3,197 ± 1,363*</td>
</tr>
<tr>
<td>Sudan III</td>
<td>1,531 ± 479*</td>
<td>1,461 ± 275*</td>
<td>12,432 ± 2,727*</td>
</tr>
</tbody>
</table>

*Significantly different from respective controls, at P < 0.05.

![Fig. 1](image1) Time course of liver enzyme activity in rats treated with 13cisRA (left) and BHT (right). QR (A), GST (B), and AHH (C) activity were measured as described in "Materials and Methods." Units of activity for QR and GST are nmol per min per mg protein and for AHH, pmol/30 min/mg protein. Points, average of 3-8 individual determinations. Control values and the appropriate SE (bars) are indicated by --- and --.--., respectively.

![Fig. 2](image2) Dose response of liver enzyme activity in rats treated with 13cisRA (left) and BHT (right). QR (A), GST (B), and AHH (C) activity were measured as described in "Materials and Methods." Units of activity for QR and GST are nmol per min per mg protein and for AHH, pmol/30 min/mg protein. Points, average of 3-8 individual determinations. Control values and the appropriate SE (bars) are indicated by --- and --.--., respectively.

from the SDS gel shown in Fig. 4, cytochromes P-450a, P-450b (or P-450d), P-450c, P-450e, P-450f (or P-450h), and P-450g were not markedly altered by retinoid treatment. Although not included as a standard on the gel, cytochrome P-450j levels (M, 51,500) also did not appear to be changed. There was, however, a slight increase in intensity of the bands corresponding to the molecular weight of cytochrome P-450g in the 13cisRA and 4HPR groups. In the 13cisRA group, a noticeable decrease in band intensity was also apparent in the molecular weight range between 50,000 and 51,000. In the retinoid-dosed groups, protein bands, not present in controls, were observed in the region of M, 60,000. BHT induced multiple changes in the electrophoretic banding pattern of microsomes. The most noticeable of these included increases in staining in the molecular weight region of cytochrome P-450e at approximately M, 65,000. However, again, fluorography showed that this region comprised a number of smaller bands. In microsomal proteins from rats that had been predosed with 13cisRA, overall NADPH-dependent binding of BaP-7,8-diol metabolites was reduced by 62%, compared with controls. This compared with a 37% reduction in binding to microsomes from animals pretested with BHT.

In Vivo Effects of Retinoid Pretreatment on Binding of BaP to Rat Tissue DNA. The binding of BaP to rat liver DNA was lower, compared to controls, in animals dosed with retinoids. Rats dosed with 13cisRA, RAc, or 4HPR had an average of 38, 27, and 40% less [3H]BaP label bound, respectively, to liver DNA (Table 3). This compared with a reduction of 34 and 32% in the BHT- and Sudan III-treated groups. Similar results were observed for stomach, with 28, 32, and 21% reductions in...
Table 2  CO-difference spectra maxima and cytochromes P-450 and b5 of microsomes of retinoid-treated male rats

Animals were dosed for 4 days with 13cisRA, RAc, 4HPR, Sudan III, and BHT at 235, 320, 800, 25, and 600 mg/kg body weight/day, respectively. The compounds were suspended in sesame oil and given p.o. Control animals received sesame oil.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CO-difference spectrum maximum</th>
<th>P</th>
<th>Cytochrome P-450 (nmol/mg microsomal protein; 5 rats/group)</th>
<th>P</th>
<th>Cytochrome b5 (nmol/mg microsomal protein; 5 rats/group)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>449.8 ± 0.46*</td>
<td>&lt;0.05</td>
<td>0.75 ± 0.17</td>
<td>0.37 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13cisRA</td>
<td>449.4 ± 0.50</td>
<td>NS*</td>
<td>0.90 ± 0.15</td>
<td>0.30 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAc</td>
<td>449.7 ± 0.54</td>
<td>NS</td>
<td>0.71 ± 0.13</td>
<td>0.26 ± 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4HPR</td>
<td>449.7 ± 0.35</td>
<td>NS</td>
<td>0.90 ± 0.15</td>
<td>0.34 ± 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudan III</td>
<td>448.8 ± 0.24</td>
<td>&lt;0.001</td>
<td>1.27 ± 0.13</td>
<td>0.52 ± 0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BHT</td>
<td>450.4 ± 0.52</td>
<td>&lt;0.01</td>
<td>0.53 ± 0.15</td>
<td>0.36 ± 0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SD; 10 determinations.
* NS, not significantly different from control (Student's t test, one tailed, P < 0.05).

We also observed 23 and 11% reductions in binding of BaP to lung DNA in animals predosed with either 13cisRA or 4HPR, respectively. Retinoids had no effect on binding of BaP to kidney DNA under the conditions described. Treatment of animals with a combination of 13cisRA and 4HPR did not cause any further reduction in binding (data not shown).

DISCUSSION

Inhibitors of carcinogenesis are divided into 3 categories according to the stage of the carcinogenic process at which they are effective (11). Retinoids have been classified as suppressing agents because they can inhibit carcinogenesis when administered after exposure to the carcinogen. There are a few reports, however, in which retinoids administered prior to the carcinogen prevented tumor formation (12–15). McCormick et al. (13) reported a significant inhibition in BaP-induced mammary carcinogenesis when retinyl acetate was administered from 2 weeks before to 1 week after the carcinogen, compared with only a temporary inhibition of tumor formation when the retinoid was administered from 1–12 weeks after the carcinogen. The authors were unable to exclude retinoid-induced alteration in BaP metabolism as the possible mechanism for tumor reduction. We have demonstrated that retinoids can cause changes in levels of important carcinogen-detoxifying enzymes. After 4 days of treatment, rats dosed with 13cisRA had elevated levels binding in the 13cisRA, RAc, and 4HPR groups, respectively (Table 4). There was an 18% reduction in the BHT-dosed group.
of QR, and those dosed with 4HPR showed elevated levels of GST. AHH levels were significantly reduced in all of the retinoid-dosed groups.

Viviani and Lutz (32) observed a strong positive correlation between microsomal AHH activity and covalent binding of BaP to rat liver DNA. In their experiments, pretreatment of Sprague-Dawley rats with MC or PB induced microsomal AHH to 380 and 160% of control and increased BaP binding to liver DNA by 175 and 190%, respectively. Similarly, we observed a retinoid-induced suppression of AHH and a corresponding reduction in binding of BaP to rat liver DNA.

Adriaenssens et al. (33) reported that dietary BHA inhibited DNA adduct formation in mouse tissues, showing inhibition in BaP binding of 45, 40, and 60%, in the forestomach, lung, and liver, respectively. In comparison, we observed 18, 23, and 34% reductions, respectively, in BaP binding to stomach, lung, and liver DNA of rats given p.o. doses of BHT.

The dose of BaP administered in our experiments was low (2 mg/kg) compared with most studies (34-37). This is important since the binding of BaP to liver DNA as a function of BaP dose is nonlinear (33, 34). In addition, BaP induces AHH, with greater induction occurring at the higher doses (34). These data suggest that retinoids would have an even greater effect at higher doses of BaP.

We cannot yet say if the isozymes responsible for the observed AHH activity are the same ones responsible for activating BaP to its ultimate carcinogen. The MC-inducible isozyme (P-450c) is not present in great amounts in normal rats since no M, 56,000 band is observed on the gels (Fig. 4). This would suggest that other isozymes are responsible for the AHH activity measured. In addition, most of the NADPH-dependent binding of the BaP-7,8-diol metabolites occurs at approximately M, 50,000 and not at M, 56,000 (Fig. 5), suggesting that reactive metabolites are being produced by different isozymes. (We assume that the reactive metabolites bind near the site of production.) Schelin et al. (37) reported similar binding of BaP-7,8-diol to the M, 50,000 region in noninduced microsomes. In addition, microsomes from MC-induced rats showed intense binding of metabolites in the region of M, 56,000, the region in which P-450c migrates. This poses a question as to which P-450 isozymes are responsible for activating polycyclic aromatic hydrocarbons in normal, noninduced animals.

We observed a 37% reduction, compared with controls, in the binding of BaP-7,8-diol metabolites to microsomal protein from BHT-dosed rats. This result agrees with studies in which the metabolism of carcinogens and binding of their metabolites to DNA were reduced in butylated hydroxyanisole- and BHT-treated rodents (38-42) and with the report of decreased production of BaP phenols and 7,8- and 9,10-diols by liver microsomes from rats fed a diet containing 1% BHT (43).

Our results appear to conflict with those of Wattenberg and Leong (44) and others (45, 46), who report that inducers of AHH activity provide a protective effect against polycyclic hydrocarbon carcinogens. The naphthoflavones and tetrachlorodibenzo-p-dioxin, for example, are AHH inducers and are also potent anticarcinogenic agents (39). Their in vivo protective effects may be due to factors other than induction of AHH, since these compounds may induce other enzymes that could detoxify carcinogens and thereby override the deleterious effects of AHH induction. Also, these compounds may themselves act as inhibitors of AHH (30, 47-49).

The reductions of AHH and BaP-7,8-biol binding may be associated with the reduced synthesis of the isozymes responsible for these activities. The reduction in activity of AHH that we observe does not, however, appear to result from a direct interaction between the enzyme and the retinoid. Retinoids do not inhibit AHH in vitro (50), and suppression of AHH does not occur when animals are dosed with near toxic levels of 13cisRA (Fig. 2). Unlike BHT and Sudan III, which induce many isozymes, retinoids may be changing metabolism by suppressing the synthesis of important isozymes.

Table 3 In vivo binding of [3H]benzo(a)pyrene to the DNA of livers in rats pretreated with retinoids or BHT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment</th>
<th>1 pmol/mg</th>
<th>2 pmol/mg</th>
<th>3 pmol/mg</th>
<th>T/C</th>
<th>T/C</th>
<th>T/C</th>
<th>Av. % reduction in binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control⁴</td>
<td>Control⁴</td>
<td>0.94</td>
<td>1.47</td>
<td>1.72</td>
<td>0.80</td>
<td>0.43</td>
<td>0.39</td>
<td>38</td>
</tr>
<tr>
<td>13cisRA</td>
<td>13cisRA</td>
<td>0.48</td>
<td>0.51</td>
<td>0.95</td>
<td>1.05</td>
<td>0.61</td>
<td>0.48</td>
<td>0.60</td>
</tr>
<tr>
<td>RAc</td>
<td>RAc</td>
<td>0.71</td>
<td>0.76</td>
<td>1.23</td>
<td>1.05</td>
<td>0.61</td>
<td>0.48</td>
<td>0.60</td>
</tr>
<tr>
<td>4HPR</td>
<td>4HPR</td>
<td>ND⁴</td>
<td>ND⁴</td>
<td>ND⁴</td>
<td>ND⁴</td>
<td>ND⁴</td>
<td>ND⁴</td>
<td>ND⁴</td>
</tr>
<tr>
<td>BHT</td>
<td>BHT</td>
<td>0.71</td>
<td>0.76</td>
<td>0.59</td>
<td>1.05</td>
<td>0.61</td>
<td>0.47</td>
<td>0.59</td>
</tr>
<tr>
<td>Sudan III</td>
<td>Sudan III</td>
<td>0.84</td>
<td>0.89</td>
<td>1.11</td>
<td>0.76</td>
<td>0.69</td>
<td>0.40</td>
<td>0.49</td>
</tr>
</tbody>
</table>

* Experiment 1 represents data obtained using livers pooled from 3 similarly treated animals; remaining determinations were obtained from single animals. Animals were treated as described in "Materials and Methods." Values for treated groups, except Sudan III, are significantly different from controls (P < 0.05) by Student's t test; control and experimental groups within each experiment were handled as matched samples.

³ T/C, treatment/control.
⁴ Control animals received 7 daily doses of sesame oil.
Microsomes from untreated male rats have been reported to contain cytochrome P-450-PCN (PB/PCN-E), which has a minimum molecular weight of 51,000 (51, 52). Treatment of rats with pregnenolone 16-α-carbonitrile results in an induction of this isozyme, accompanied by a 3-fold increase in microsomal AHH activity (53). It is possible that the reduction in AHH activity and the decrease in the M, 51,000 band following 13cisRA treatment are reflections of a decrease in the constitutive level of cytochrome P-450-PCN (PB/PCN-E). Another possible candidate for the observed reduction in AHH is P-450h, which has good AHH activity in noninduced rats (54). The shift in the CO-reduced difference spectrum towards 448 nm could be explained by the apparent increase in cytochrome P-450g, since the maximum for this purified isozyme is at 447.5 nm (54).

The levels of individual P-450 isoforms can vary greatly depending on the dose and the type of chemical to which an animal is exposed. In addition, species, type of diet, and nutritional status of the animal (i.e., fasting) are also important (55–58). The sensitivity of mixed-function oxidases to changes in environment could explain the variations observed among laboratories. Leo et al. (16), for example, reported that feeding rats 100 times the normal amount of RA for 2 weeks resulted in an increased hepatic cytochrome P-450 content. They also reported a shift toward 449 nm in the CO-reduced difference spectra and, on SDS gel electrophoresis, an increase in a band of minimum molecular weight of 51,000 (51, 52). Treatment of rats with pregnenolone 16-α-carbonitrile results in an induction of this isozyme, accompanied by a 3-fold increase in microsomal PON activity (11). Retinoids are as good as or better than either BHT or Sudan III in reducing BaP binding to DNA of rat liver and target tissue, i.e., stomach. This effect suggests that retinoids would also inhibit initiation of tumor formation of carcinogens requiring metabolic activation. Evidence for this should come from additional in vivo experiments.

The inductive effects produced by blocking agents usually result in enhanced carcinogen detoxification but may also lead to increased carcinogen activation (11). This finding may explain why, under certain conditions, retinoids and BHT enhance tumor development (61–64). Tissue-specific and dose-dependent changes in metabolizing enzymes need to be investigated so that differences among retinoids can be exploited for developing more active chemopreventive agents.

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