Inhibition by 6-Mercaptopurine of the Binding of a Benzo(a)pyrene Diol-Epoxide to DNA in Chinese Hamster Ovary Cells

Michael C. MacLeod, Ronald M. Humphrey, Tim Bickerstaff, and Anne Daylong

Department of Experimental Carcinogenesis, Science Park-Research Division, University of Texas System Cancer Center, Smithville, Texas 78957

ABSTRACT

The finding that 7,8,9,10-tetrahydrobenzo(a)pyrene (BPDE-I) is stabilized against hydrolysis by binding to cellular membranes suggested that nucleophilic compounds which would colocalize with BPDE-I in membranes might inhibit the deleterious biological effects of BPDE-I. We have explored the possibility that hydrophobic, sulfhydryl-containing compounds might provide such inhibition using the binding of BPDE-I to DNA in Chinese hamster ovary cells as a biological end point. Of several compounds tested, 6-mercaptopurine (6-MP) was the most potent, exhibiting 50% inhibition of BPDE-I:DNA binding at about 30 μM and about 95% inhibition at 500 μM. 6-MP, at concentrations of 30 μM or greater, was also effective in preventing the induction of mutations by BPDE-I at the aprt locus. By varying the time of addition of the two compounds, it was shown that the action of 6-MP is intracellular. In vitro, 6-MP readily forms an adduct with BPDE-I, and the same adduct is found as a major metabolite in cells treated with BPDE-I and 6-MP. These findings are consistent with the hypothesis that 6-MP and BPDE-I colocalize in membranes of Chinese hamster ovary cells and form a covalent adduct, thus preventing the BPDE-I from interacting with critical cellular macromolecules such as DNA. Several nontoxic derivatives of 6-MP (9-methyl-6-MP, 2,6-dithiopurine) or analogues of 6-MP (4-mercapto-1H-pyrazolo[4,3-d]pyrimidine) were also tested in the Chinese hamster ovary cell system and found to inhibit binding of BPDE-I to DNA with potencies comparable to that of 6-MP.

INTRODUCTION

Many chemical carcinogens are thought to exert their deleterious effects through the production of electrophilic intermediates which modify cellular macromolecules (1, 2). For a number of polycyclic aromatic hydrocarbons, including the well-studied model compound B(a)P, the metabolic pathway which leads to the major DNA-binding electrophile has been established (3, 4). Several enzymatic steps, involving oxygenation by the cytochrome P-450 system and hydration by epoxide hydrolase, lead to the production of bay region diol-epoxides, which are thought to be the ultimate carcinogenic derivatives of their parent hydrocarbons (3–7). In particular, BPDE-I, a bay region diol-epoxide derived from B(a)P, is responsible for most DNA binding in cells treated with B(a)P and is also the most potent metabolite of B(a)P as a mouse skin carcinogen (8–13). It would therefore be useful to find ways to prevent the reaction of BPDE-I with critical cellular targets as a way of intervening in the carcinogenic process.

Although glutathione, a major endogenous cellular nucleophile, reacts only slowly with BPDE-I (14), several glutathione S-transferases have been shown to catalyze conjugation of BPDE-I to the sulfhydryl moiety of glutathione (14). In intact hepatocytes, this pathway is effective in detoxifying BPDE-I, where the glutathione conjugate represents the major intracellular metabolite of BPDE-I (15). In fact, the high level of glutathione S-transferase activity in liver and the concomitant efficiency of BPDE-I detoxification have been suggested to be one of the reasons for the lack of carcinogenicity of B(a)P in normal, adult rat liver (16). However, other tissues which are targets for B(a)P carcinogenesis, e.g., mouse skin and rat mammary gland, have lower levels of this glutathione S-transferase (16).

The high rate of glutathione conjugation in hepatocytes apparently causes an increased rate of disappearance of BPDE-I from hepatocyte culture medium (15), compared to the spontaneous hydrolysis rate seen in aqueous medium. In contrast to this behavior, we have recently found that BPDE-I added to the culture medium of CHO cells rapidly enters a hydrophobic, subcellular fraction in which it is protected from hydrolysis (17). Glutathione conjugates are apparently not formed in these cells. Dock et al. (15) have reported similar stabilization of BPDE-I by rat liver microsomes and liposomes prepared from microsomal lipids, supporting the hypothesis that BPDE-I tends to localize in membrane fractions in intact cells (17). Indeed, DNA binding studies in a variety of systems have suggested a relatively long biological half-life for BPDE-I (18-21). These findings suggested to us that an attractive chemical intervention strategy would be to find a highly reactive nucleophile which was also hydrophobic and would therefore tend to colocalize with BPDE-I intracellularly. Such a compound could then act as a scavenger in cells which lack high levels of glutathione and glutathione S-transferase, reacting covalently with BPDE-I molecules before significant interaction with cellular macromolecules took place. The fast rate of reaction between BPDE-I and the ionized form of 2-mercaptoethanol (22) suggested the use of thiol compounds as candidate nucleophiles. Using two bioassays, the binding of radioactive BPDE-I to DNA in intact CHO cells and the induction of mutations at the aprt locus in the same cells, we have tested several sulfhydryl compounds of varying polarities as potential inhibitors. We present here these data and further characterization of the mode of action of one active compound, 6-MP.

MATERIALS AND METHODS

Cells and Labeling Conditions. Cultures of AT3-2 cells, a subline of CHO cells obtained from Dr. G. Adair, were maintained, treated with 0.5 or 1.0 μM [3H]BPDE-I for 60 min (unless otherwise stated), and analyzed for covalent binding to DNA as described previously (17). Test compounds were initially dissolved in ethanol and added to cultures 5 min prior to addition of BPDE-I; all treatments were in serum-free media and the final ethanol concentration did not exceed 1% which is nontoxic to these cells. To obtain a more complete dose response for 6-MP, a concentrated stock was prepared in 1.0 N NaOH. Treatments over the entire dose range were made using appropriate dilutions such that the final concentration of added NaOH in the medium was 0.001 N in all cases. For cytotoxicity assays, cells were treated with the test compound without BPDE-I, then harvested by trypsinization, and replated at low density to measure plating efficiency. There was no significant difference between the plating efficiencies of solvent only and untreated controls.
Analysis of Mutant Frequency. CHO cells were pretreated for 5 min with various concentrations of 6-MP and then 0.5 μM unlabeled BPDE-I was added as described for the DNA labeling experiments. For each concentration of 6-MP, triplicate plates of cells were treated with BPDE-I for determination of mutant frequency and a fourth plate was treated for determination of cell survival. After 60 min of incubation, cell monolayers were washed twice with growth medium and incubated overnight in growth medium. Appropriate numbers of cells from each treatment plate were subcultured in triplicate to give a total expression time of 7 days and mutant frequency was then determined by selection in medium containing 8-azauridine (23).

Analysis of BPDE-I-6-MP Adducts. Adducts were formed in vitro by reaction of 6-MP and [3H]BPDE-I in 10 mM Tris, pH 7.4, at 22°C for 1 h. Adducts formed intracellularly were extracted from the washed cell monolayers with methanol (17). The putative adducts could in both cases be extracted into ethyl acetate, along with tetrathionate products of BPDE-I, and analyzed by HPLC as indicated in the figure legends.

Chemicals. Thiocresol, thiophenol, mercaptopyrimidine, 6-MP, 2-mercaptoethanol, and mercaptoethanesulfonic acid were the highest purity available from either Aldrich (Milwaukee, WI) or Sigma (St. Louis, MO) and were used without further purification. Samples of 2-chloro-6-MP, 1-methyl-4-nitro-5-thioimidazole, 9-methyl-6-MP, 2-OH-6-MP, 2,6-dithiopurine, and 4-mercaptopurine were generously provided by the Wellcome Research Laboratories. [3H]BPDE-I was obtained from the National Cancer Institute Chemical Carcinogen Standard Repository. Stock solutions (either 100 or 200 μM) were prepared in absolute ethanol and stored at −20°C. Concentrations were verified by absorbance and fluorescence spectrophotometry (24).

RESULTS

Inhibition of DNA Binding by Aromatic Sulfydryl Compounds. A series of sulfur-containing compounds were tested for their ability to inhibit BPDE-I-DNA binding in CHO cells at several different concentrations. Three nonaromatic, sulfur-containing compounds, allyl sulfide, 2-mercaptoethanol, and mercaptoethanesulfonic acid, were tested because they had previously been shown to be either good electrophile scavengers (22, 25) or had demonstrated anticarcinogenic activity (26). At concentrations up to 100 μM, these compounds did not significantly inhibit BPDE-I-DNA binding in this intact cell assay (data not shown). However, as can be seen in Table 1, two aromatic sulfydryl compounds, thiocresol and thiophenol, and two heterocyclic, nucleobase analogues, mercaptopyrimidine and 6-MP, demonstrated significant inhibitory activity, at least at the highest concentration. The highest level of inhibition, 66%, was obtained with 50 μM 6-MP, and this compound was selected for further evaluation.

Action of 6-MP. A more complete dose-response curve for 6-MP inhibition of BPDE-I-DNA binding is shown in Fig. 1. Above a concentration of about 1 μM, the fractional inhibition increased with the log of the 6-MP concentration, giving approximately 50% inhibition at about 30 μM 6-MP. At the highest dose tested (500 μM), inhibition was greater than 95%. 6-MP has been used as a cytotoxic, chemotherapeutic agent in leukemia treatment (27), where it is thought to act through incorporation into cellular nucleic acids (28). To test whether such effects were important in its inhibition of BPDE-I-DNA binding, we added equimolar amounts of inosine which should block the purine metabolism-related cytotoxic effects of 6-MP. As shown in Fig. 1 (C) this had no effect on the inhibition of binding by 6-MP. In fact, plating efficiency assays (see below, Table 3) demonstrated virtually no toxicity to CHO cells under our treatment conditions over the effective range of 6-MP concentrations; this was true in the presence or absence of inosine. The lack of toxicity may be due to the short exposure time used in these assays. We conclude that the mechanism of action of 6-MP in this assay is not dependent on cytotoxic effects mediated by incorporation into nucleic acid.

Since several compounds are known which detoxify BPDE-I by increasing the rate of its hydrolysis in aqueous solvents (29–31), it seemed possible that 6-MP could be inhibiting BPDE-I binding by catalyzing detoxification extracellularly, in the labeling medium. To test this, we altered the order of addition of the compounds to the cultures. We first incubated CHO cells with BPDE-I for 15 min; based on our previous studies (17) at this time point there is very little intact BPDE-I remaining in the medium. There is, however, a significant pool of unhydrolyzed, extracellular BPDE-I which continues to bind to DNA in a time-dependent manner for at least a further 75 min (17). If the 6-MP effect were due to extracellular hydrolysis, addition of 6-MP after 15 min of BPDE-I treatment would have no effect on the subsequent increase in DNA binding, since the extracellular BPDE-I has already been hydrolyzed. However, if the 6-MP effect is due to an interaction with the intracellular pool of BPDE-I, then one might expect an inhibition of the increase in DNA binding occurring between 15 and 60 min of incubation. As shown in Table 2, without 6-MP treatment the specific activity of the DNA increased by about 50% between 15 and 60 min of BPDE-I treatment; this increase was statistically significant (P < 0.01). Addition of 100 μM 6-MP at 15 min completely abolished this increase in DNA binding (Table 2, Lines 1 and 3). This strongly suggests that 6-MP in some way prevents the reaction of intracellular BPDE-I with DNA.

Inhibition of Mutagenesis. We have recently shown that mutations are induced at the aprt locus in CHO cells by treatment with BPDE-I and that the mutant frequency increases linearly with adduct formation at low doses (23). Accordingly, one would expect that mutagenesis would be inhibited in cells

Table 1 DNA binding inhibition by aromatic sulfur compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>DNA binding</th>
<th>Normalized DNA binding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(control (0 μM) (pmol/mg DNA)]</td>
<td>(fraction of control)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 μM</td>
</tr>
<tr>
<td>Thiocresol</td>
<td>146.6 ± 12.5</td>
<td>1.11 ± 0.02</td>
</tr>
<tr>
<td>Thiophenol</td>
<td>172.6 ± 4.1</td>
<td>0.79 ± 0.08</td>
</tr>
<tr>
<td>Mercaptopyrimidine</td>
<td>147.2 ± 8.6</td>
<td>1.04 ± 0.003</td>
</tr>
<tr>
<td>6-MP</td>
<td>127.7 ± 30.4</td>
<td>0.67 ± 0.25</td>
</tr>
</tbody>
</table>

* CHO cells were treated with solvent only (0.001 N NaOH) for 5 min, then 1.0 μM [3H]BPDE-I was added for 1 h, and DNA binding was determined as described (17). Briefly, DNA was purified by hydroxyapatite fractionation and solvent extraction from three replicate cultures of cells, and the specific activity (pmol BPDE-I bound/mg DNA) was determined by liquid scintillation counting and spectrophotometry.

* CHO cells were treated with the indicated concentration of test compound for 5 min; then 1.0 μM [3H]BPDE-I was added for 1 h. DNA binding was quantitated as described above. Specific activities in three replicate cultures were determined and normalized to the mean specific activity determined in the control cultures ([3H] BPDE-I only) in the same experiment.

* Mean ± SD.

* ND, not determined.
6-MERCAPTOPURINE INHIBITION OF BPDE-DNA BINDING

Fig. 1. Inhibition of BPDE-I:DNA binding by 6-MP. CHO cells were preincubated for 5 min with the indicated concentration of 6-MP (MPU). Then, [3H]BPDE-I was added to 1.0 µM and the incubation was continued for 60 min. DNA was harvested and purified from the plates as described (17), and the specific activity was determined and normalized to that determined with no added 6-MP. ○, 6-MP dissolved in NaOH, n = 9; Δ, 6-MP dissolved in ethanol, n = 7; O, 6-MP + equimolar inosine dissolved in ethanol, n = 3. Bars, SD.

Table 2 Effect of posttreatment with 6-MP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normalized DNA binding*</th>
<th>Significance (P) vs. 15-min treatment**</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPDE-I, 15 min</td>
<td>1.0 ± 0.19</td>
<td>2 &gt;0.8</td>
</tr>
<tr>
<td>BPDE-I, 15 min; BPDE, 45 min</td>
<td>1.53 ± 0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BPDE-I, 15 min; 6-MP + BPDE, 45 min</td>
<td>1.03 ± 0.10</td>
<td>&gt;0.8</td>
</tr>
</tbody>
</table>

*CHO cells were treated with 0.5 µM [3H]BPDE-I for 15 min. One-third of the cultures were harvested immediately, one-third were incubated for a further 45 min with no further addition, and one-third were incubated for a further 45 min after adding 6-MP to the BPDE-I-containing medium to a final concentration of 100 µM. DNA specific activities were determined in three replicate cultures for each treatment group and normalized to that of the 15-min treatment.

**The significance of the difference from the specific activity for the 15-min treatment group was determined using Student’s t test.

Table 3 Inhibition of BPDE-I-induced mutagenesis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plating efficiency (%)</th>
<th>Mutant frequency (mutants/10⁶ survivors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPDE-I</td>
<td>6-MP (µM)</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>0</td>
<td>71.2 ± 5.5</td>
</tr>
<tr>
<td>–</td>
<td>3</td>
<td>64.8 ± 6.9</td>
</tr>
<tr>
<td>–</td>
<td>30</td>
<td>63.9 ± 19.5</td>
</tr>
<tr>
<td>–</td>
<td>300</td>
<td>63.1 ± 10.5</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>11.9 ± 0.6</td>
</tr>
<tr>
<td>+</td>
<td>3</td>
<td>15.8 ± 1.7</td>
</tr>
<tr>
<td>+</td>
<td>10</td>
<td>24.0 ± 1.5</td>
</tr>
<tr>
<td>+</td>
<td>30</td>
<td>55.5 ± 2.7</td>
</tr>
<tr>
<td>+</td>
<td>100</td>
<td>72.8 ± 2.1</td>
</tr>
<tr>
<td>+</td>
<td>300</td>
<td>57.6 ± 6.2</td>
</tr>
</tbody>
</table>

*Mutants at the aprt locus were selected by growth in medium containing 8-azaadenine as described (22).

**Cytotoxicity was determined by plating efficiency assays.

Table 3 Inhibition of BPDE-I-induced mutagenesis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plating efficiency (%)</th>
<th>Mutant frequency (mutants/10⁶ survivors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPDE-I</td>
<td>6-MP (µM)</td>
<td></td>
</tr>
<tr>
<td>–</td>
<td>0</td>
<td>71.2 ± 5.5</td>
</tr>
<tr>
<td>–</td>
<td>3</td>
<td>64.8 ± 6.9</td>
</tr>
<tr>
<td>–</td>
<td>30</td>
<td>63.9 ± 19.5</td>
</tr>
<tr>
<td>–</td>
<td>300</td>
<td>63.1 ± 10.5</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>11.9 ± 0.6</td>
</tr>
<tr>
<td>+</td>
<td>3</td>
<td>15.8 ± 1.7</td>
</tr>
<tr>
<td>+</td>
<td>10</td>
<td>24.0 ± 1.5</td>
</tr>
<tr>
<td>+</td>
<td>30</td>
<td>55.5 ± 2.7</td>
</tr>
<tr>
<td>+</td>
<td>100</td>
<td>72.8 ± 2.1</td>
</tr>
<tr>
<td>+</td>
<td>300</td>
<td>57.6 ± 6.2</td>
</tr>
</tbody>
</table>

mutants/survivor. The data presented in Table 3 indicate an inhibition of BPDE-I-induced mutagenesis at the aprt locus by 6-MP at concentrations of 30 µM or greater. The reduction in mutant frequency was statistically significant at concentrations between 30 and 300 µM (Student’s t test, P < 0.01) and amounted to about an 87% reduction in induced mutations at 300 µM 6-MP ([6.2 × 10⁻⁵ - 3.7 × 10⁻⁵]/(23.4 × 10⁻⁵ - 3.7 × 10⁻⁵)). These concentrations also inhibited BPDE-I-induced cytotoxicity (Table 3).

Analysis of 6-MP:BPDE-I Adducts. Since sulphhydril-containing compounds are known to be reactive with diol-epoxides (22), we hypothesized that the reaction of 6-MP with BPDE-I mediated its inhibition of DNA binding. To test one prediction of this hypothesis we analyzed methanol extracts of cells for possible BPDE-I:6-MP adducts. As a standard, we allowed BPDE-I to react with 6-MP in aqueous solution and analyzed the reaction products by HPLC. As can be seen in Fig. 2B, an unidentified peak (tR = 14.1 min) was present in the HPLC profile in addition to peaks representing tetrol hydrolysis products (compare to Fig. 2A; no 6-MP in reaction mixture). The appearance of this peak was absolutely dependent on the presence of 6-MP in the reaction mixture, and its abundance relative to the tetrots increased with increasing 6-MP concentration (data not shown). Absorbance spectroscopy demonstrated the existence of a pyrene-like chromophore in this material (data not shown) but with absorption maxima shifted approximately 10 nm towards longer wavelengths; similar behavior has been seen with a 2-mercaptopentanoyl adduct of BPDE-I (17, 22). We tentatively identify this product as a BPDE-I:6-MP adduct; further chemical characterization of this product will be presented elsewhere. 4 Our predicted mode of action of 6-MP suggests that an adduct of BPDE-I:6-MP should be formed in...
cells treated with the two compounds, and such an adduct might be recoverable from the methanolic extract of treated cells. In fact, several compounds were noted in the HPLC profiles of the cellular extracts (Fig. 2C); of these, the major one was cochromatographic with the in vitro putative BPDE-I:6-MP edge that sulfhydryl compounds react readily with BPDE-I (24).

Chemoprevention strategies aimed at each of these key processes. In the present investigation, we have combined the knowledge of BPDE-I-induced biological damage (Step 3 of the scheme of Fig. 1). These processes are often at the cellular level where intervention would be possible (Fig. 1). As discussed by these authors, research is ongoing to develop chemoprevention strategies aimed at each of these key processes. In the present investigation, we have combined the knowledge that sulfhydryl compounds react readily with BPDE-I and intracellular BPDE-I implies that the two pools are not in rapid equilibrium. Again, the appearance of the major product was absolutely dependent on the presence of 6-MP in the treatment protocol.

Inhibition of DNA Binding by 6-MP Analogues. Several analogues and derivatives of 6-MP have been reported to be nontoxic when injected into mice (32). Several such nontoxic analogues which retain at least one thiol moiety were tested for inhibition of the binding of BPDE-I to DNA in CHO cells (Table 4). Of the compounds tested, 2-chloro-6-MP and 1-methyl-4-nitro-5-thiomimidazole showed inhibitory activities that were clearly lower than that of 6-MP. The remaining 5 compounds (9-methyl-6-MP, 2-methyl-6-MP, 2-OH-6-MP, 2,6-dithiopurine, and 4-mercapto-1H-pyrazolo[3,4-d]pyrimidine) had inhibitory activity comparable to or greater than that of 6-MP (Table 4).

**DISCUSSION**

One of the important long-term aims of carcinogenesis research is to develop strategies for the prevention of cancer. In a recent review and position paper, Bertram et al. (33) have identified 6 known processes in the chemical carcinogenesis pathway at which intervention would be possible (Fig. 1). As discussed by these authors, research is ongoing to develop chemoprevention strategies aimed at each of these key processes. In the present investigation, we have combined the knowledge that sulfhydryl compounds react readily with BPDE-I and recent findings (15, 17) suggesting that BPDE-I partitions into cellular membranes where it is stabilized against hydrolysis, to suggest a new class of compounds as intracellular inhibitors of BPDE-I-induced biological damage (Step 3 of the scheme of Bertram et al.). As a result of our initial evaluation of this suggestion, we find that 6-MP is an effective inhibitor of BPDE-I:DNA binding in CHO cells. As expected, there is a concomitant inhibition of BPDE-I-induced mutagenesis (Table 3). The effective range of 6-MP treatments does not induce measurable cytotoxicity or mutagenesis in the absence of BPDE-I. Presumably, this lack of cytotoxic effects is due to the short (~1 h) treatment time utilized. The experiment presented in Table 2 suggests that the effect of 6-MP is intracellular, since 6-MP addition at a time when there is very little extracellular BPDE-I is effective in blocking further binding of intracellular BPDE-I. In addition, the less hydrophobic sulfhydryl compounds, 2,6-dithiopurine, and 2,6-dithiopurine, show no detectable activity. The high level of the putative 6-MP:BPDE-I adduct found in either in vitro reactions or in CHO cells (Fig. 2) is also consistent with a “scavenging” mode of action for 6-MP.

In this respect, 6-MP is similar to several other compounds, including riboflavin 5'-phosphate and ellagic acid (30, 31), which exhibit rapid rates of reaction with BPDE-I. Although a more detailed description of the reaction between 6-MP and BPDE-I will be presented elsewhere (4), the fact that at pH 7.4 the 6-MP adduct is formed in higher amounts than are tetracycline products (Fig. 2, A and B) indicates that the rate constant for the 6-MP reaction must be higher than those for spontaneous and acid-catalyzed hydrolysis. In fact, the product ratios are similar to those reported for ellagic acid, an extremely potent inhibitor of BPDE-I-induced mutagenesis (31).

It remains for further experimentation to demonstrate whether 6-MP or other related aromatic sulfhydryl compounds exhibit protective activity against the carcinogenic activity of BPDE-I or its parent compound B(a)P. Although several plant phenols, notably ellagic acid, are extremely effective in inhibiting BPDE-I mutagenesis (31), in vivo activity against BPDE-I carcinogenesis is only moderate, and no inhibitory activity against B(a)P could be demonstrated (34). However, as noted by Chang et al. (34), the intracellular concentration and lifetime of the inhibitor are certain to be important parameters in inhibition of biological effects. Thus, the pharmacokinetics of a potential inhibitor is at least as important as its in vitro reaction rate with BPDE-I. In this respect, 6-MP may have an important advantage since it is a good analogue of purine. As such, it is actively transported into cells by the purine transport system (35), and this may act to increase its intracellular concentration. This may also explain why it is a more potent inhibitor than thioreosol or thiophenol, which should partition into membranes as well as or better than 6-MP but would not be expected to be actively transported. The known cytotoxic action of 6-MP (27, 28) represents a significant barrier to its use as a chemopreventive agent in vivo. However, several analogues which are nontoxic in mice have been described (32), and the initial tests (Table 4) indicate good inhibitory activity for several of these compounds, including 9-methyl-6-MP, 4-mercapto-1H-pyrazolo[3,5-d]pyrimidine, and 2,6-dithiopyrimidine, in the CHO cell BPDE-I:DNA binding assay. The possibility that these nontoxic 6-MP analogues may inhibit BPDE-I-induced tumorigenesis in vivo is currently being tested.

**ACKNOWLEDGMENTS**

We thank J. Mayhugh, J. Riley, and J. Ing for help in manuscript preparation and V. Coy for technical assistance. We also thank Beverly Sullivan, Gertrude Elion, and Tom Zimmerman for helpful discussions and for assistance in obtaining the 6-MP analogues.

**REFERENCES**

6. Yang, S. K., McCourt, D. W., Roller, P. P., and Gelboin, H. V. Enzymatic
conversion of benzo(a)pyrene leading predominantly to the diol-epoxide r-7,8-dihydroxy-9,10-oxo-7,8,9,10-tetrahydrobenzo(a)pyrene through a single enantiomer of r+7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene. Proc. Natl. Acad. Sci. USA, 73: 2594-2598, 1976.


Inhibition by 6-Mercaptopurine of the Binding of a Benzo(a)pyrene Diol-Epoxide to DNA in Chinese Hamster Ovary Cells

Michael C. MacLeod, Ronald M. Humphrey, Tim Bickerstaff, et al.


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/50/14/4355

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.