Use of a microsome-mediated test system to assess efficacy and mechanisms of cancer chemopreventive agents

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There is a growing need for short-term assays which can assess the mechanisms and efficacy of cancer chemopreventive agents. In the present study we have employed a microsome-mediated test system concomitantly with DNA adduct detection to assess the efficacy of five chemopreventive agents, N-acetylcysteine, butylated hydroxytoluene (BHT), curcumin, oltipraz, and ellagic acid. 32P-Postlabeling analysis of DNA incubated with benzo[a]pyrene (BP) in the presence of Aroclor 1254-induced microsomes produced two major adducts: one derived from the interaction of benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) with deoxyguanosine (dG) and the other from further activation of 9-OH-BP (309 and 34 adducts/106 nucleotides, respectively). With the exception of N-acetylcysteine, all test agents significantly altered BP–DNA adduct levels: Intervention with ellagic acid and oltipraz substantially inhibited both BPDE–dG and 9-OH-BP adducts, while intervention with curcumin and BHT inhibited the BPDE–dG adduct (both 57% and 38%, respectively) and enhanced the 9-OH-BP adduct (230% and 650%, respectively). Furthermore, ellagic acid was the only test agent observed to inhibit the anti-BPDE–dG adduct in the absence of microsomal enzymes, which is consistent with the known conjugation of ellagic acid with BPDE. These results suggest that oltipraz may be acting as an inhibitor of P4501A1, the isozyme involved in activation of BP to BPDE, or by conjugation of the electrophilic species by a metabolite of oltipraz. A plausible mechanism for inhibition of the BPDE–dG adduct and enhancement of the 9-OH-BP adduct by curcumin and BHT includes inhibition of epoxide hydrolase. Our results also indicate that N-acetylcysteine does not act as an electrophilic trapping agent of BP metabolites but may exert its protective effect in vivo by various other means, including modulation of detoxification enzymes and altering DNA repair processes. These data suggest that this cell-free system in conjunction with the sensitive 32P-postlabeling DNA adduct analysis may prove a viable test system for assessing the mechanisms and efficacy of chemopreventive agents.

Introduction

Chemoprevention is a rapidly growing field in cancer research which is aimed at inhibiting or delaying the onset of carcinogenesis. Over 500 compounds have been identified with potential chemopreventive properties as a result of their ability to inhibit tumor formation or cell transformation (1). The multifactorial nature of the carcinogenesis process provides numerous pathways (2,3) which may be manipulated by these agents thus resulting in inhibition or delaying of tumor development (4). These pathways include metabolic activation of a procarcinogen to its ultimate carcinogenic form, detoxification of the mutagenic compound, direct interaction of the preventive agent with the carcinogenic species, antioxidant, DNA repair processes and cell proliferation. Due to the multiplicity of suspected chemopreventive agents, short-term screening assays are currently being developed which will assist in the identification of efficacious compounds (5).

Traditional biomarkers employed in chemoprevention studies involve animal bioassays having endpoints, including tumor formation and multiplicity, and the development of premalignant lesions. These assays are often time consuming and expensive thereby limiting the number of agents that can be tested in a reasonable time-frame. Recent studies have used endpoints such as alteration of phase II enzyme levels, including glutathione transferases, glucuronyl transferases, and sulfo-transferases which are often responsible for detoxifying carcinogens; inhibition of free-radical formation; poly(ADP-ribose)polymerase inhibition, believed to be involved in DNA repair and cell differentiation, oncogene and tumor suppressor gene expression; alteration of DNA methylation patterns; and modulation of tyrosine kinase, protein kinase C and ornithine decarboxylase levels which have been associated with cell proliferation and terminal differentiation (4,5).

The use of relevant biochemical markers of carcinogenesis is intrinsic to developing successful screening of chemopreventive agents (4,6). These endpoints should represent a significant step in the process of carcinogenesis and provide some criteria for distinguishing efficacious compounds from ineffective ones. Since no single endpoint can accurately represent the entire process of cancer development it may be necessary to study a variety of endpoints each assessing different stages of this disease process. One applicable genomic biomarker is DNA adduction. DNA adduct formation is a prerequisite for carcinogenesis as shown for the majority of known carcinogens (7) and represents the net balance between activation and detoxification of these agents (8). They can be measured rapidly both in vitro and in vivo and have been well documented for many carcinogens (3).

In this study a microsomal assay concomitant with DNA adduction has been employed as a potential screening method to assess the ability of five suspected or known anticarcinogenic compounds, curcumin, oltipraz, butylated hydroxytoluene (BHT*), N-acetylcysteine and ellagic acid, to alter the binding of benzo[a]pyrene (BP) to DNA. BP is a ubiquitous environmental carcinogen which induces skin, lung (9) and mammary tumors in rodents (10). This potent carcinogen requires bioactivation to its ultimate carcinogenic

*Abbreviations: BHT, butylated hydroxytoluene; BP, benzo[a]pyrene; BPDE, benzo[a]pyrene-7,8-diol-9,10-epoxide; dG, deoxyguanosine; PAH, polycyclic aromatic hydrocarbon; PEI-cellulose, polyethyleneimine-cellulose; EH, epoxide hydrolase; RAL, relative adduct level.
form by cytochrome P450 enzymes (9) which are localized predominantly in the endoplasmic reticulum and concentrated in the liver although other tissues also contain these enzymes but in lesser amounts (11). For these reasons this BP/rat liver microsomal system was chosen to study chemopreventive agents and their mechanistic properties.

The five chemopreventive agents studied were selected based on their known or proposed multiple protective mechanisms and their anticarcinogenic effects in rodent models. BHT is a synthetic antioxidant which has been shown to have antitumorigenic actions in a variety of rodent tissues, including liver, mammary gland, skin and stomach, and against chemically diverse carcinogens, including BP, azoxymethane and 2-acetylaminofluorene, however, BHT has also been shown to enhance tumor yield in some models (12). The chemoprotective effect of BHT is associated with its ability to increase tissue glutathione levels, inhibition of lipid peroxidation and inhibition of metabolic activation of carcinogens. Ellagic acid also has antioxidant properties while its chemopreventive activity has largely been associated with its inhibition of cytochrome P450s and its ability to directly bind the activated form of polycyclic aromatic hydrocarbons (PAHs) such as BP: it has been shown to inhibit lung, skin, colon and bladder tumorgenesis in animal models (reviewed in 13). Curcumin has multiple chemoprotective properties including antioxidant and anti-inflammatory activity, inhibition of the arachidonic acid pathway and inhibition of carcinogen bioactivation. These properties may all contribute to its chemopreventive activity observed in mouse skin, rat mammary and mouse colon (13). Oltipraz, a synthetic dithiolthione, has been shown to protect against lung, liver, trachea, breast, skin, bladder and colon carcinogenesis in rodent models (13) (reviewed in 14). Its chemopreventive properties are primarily associated with its ability to induce enzymes involved in maintenance of reduced glutathione pools and those involved in electrophilic detoxification including glutathione-S-transferase and epoxide hydrolase (EH), respectively. Oltipraz has also been observed to have antiproliferative properties which may also contribute to its chemoprotective ability (13,14). N-Acetylcysteine also enhances reduced glutathione levels, DNA-repair processes and detoxification enzymes (reviewed in 15). N-Acetylcysteine, like these other chemopreventive agents, has chemoprotective effects in a variety of organs including trachea, mammary, colon and bladder (13).

Materials and methods

Chemicals

Oltipraz was generously provided by Dr. Vernon E. Steele of the National Cancer Institute Chemoprevention Investigators Branch, Aroclor 1254 and anti benz[a]pyrene-7,8-diol-9,10-epoxide (anti BPDE) were respectively provided by Dr. Larry Robertson of the University of Kentucky, Lexington, KY and Dr. Subodh Kumar of the State University New York College, Buffalo, NY. Curcumin (95%) was purchased from Janssen Chimica (New Brunswick, NJ). Ellagic acid (95%), BHT, BP (98%), NADP+, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Co. (St Louis, MO). N-Acetyl-l-cysteine (98%) was from Aldrich Chemical Co. (Milwaukee, WI). Sources of all chemicals used for 32P-postlabeling analysis were as described previously (16).

Preparation of Aroclor 1254-induced rat liver microsomes

Four, 6-week-old (150-174 g) male Sprague-Dawley rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN), housed in metal cages and allowed to acclimatize for 7 days prior to treatment. Food and water were supplied ad libitum throughout the experiment. The animals were injected intraperitoneally with a single dose (500 mg/kg body weight) of Aroclor 1254. Rats were killed 4 days post-dosing by cervical dislocation. Each liver was perfused in situ with 0.1 M sodium phosphate buffer/1.15% NaCl (pH 7.6). Excised and microsomes prepared as described (17) with minor modifications: whole liver homogenates were prepared in sodium phosphate buffer/1.15% NaCl (pH 7.6), the microsomal pellet was washed with 10 mM EDTA/1.15% NaCl and reconstituted in 0.25 M sucrose. Microsomal protein was assayed by the method of Lowry et al. (18).

In vitro microsomal assay

The chemopreventive agents (10 μM, 50 μM, or 150 μM) or vehicle alone were preincubated at room temperature with calf thymus DNA (300 μg/ml), 1 mg/ml Aroclor 1254-induced microsomal protein, 1 mM MgCl2, 2.5 mM glucose-6-phosphate, 1 unit/ml glucose-6-phosphate dehydrogenase, 0.5 mM NADP+ in 1 ml 50 mM Tris-HCl (pH 7.5). After incubation for 10 min. BP (10 μM or 20 μM) was added to the above cocktail and the incubation continued in a shaking water-bath. The reaction was terminated after 30 min by addition of 20 mM EDTA, followed by centrifugation (7500 r.p.m., 10 min) to discard the microsomal pellet. The carcinogens and chemopreventive agents were freshly dissolved in dimethyl sulfoxide (final concentration 1.5%). DNA was isolated from the supernatant by removal of RNA and proteins by digestion with RNAses A and T1 and proteinase K, respectively, and solvent extractions, followed by precipitation of the DNA with ethanol (16). The DNA concentration was estimated spectrophotometrically using 1 A260 unit equals 50 μg DNA.

In vitro non-microsomal assay

The chemopreventive agents (150 μM) or vehicle alone were preincubated at room temperature for 10 min in the presence of calf thymus DNA (100 μg/ml) and 5 mM sodium succinate (pH 6.0). Anti BPDE (1 μM) was added to the cocktail in a shaking water-bath. The reaction was terminated after 30 min by extracting twice with water-saturated ethyl acetate, followed by extraction with Sevag (chloroform/isooamyl alcohol, 24:1). After jophosphilizing the DNA was analyzed by 32P-postlabeling. BPDE and the chemopreventive agents were freshly dissolved in dimethyl sulfoxide (final concentration 2%).

Analysis of DNA adducts

Adducts were analyzed by the nuclease P1-version of the 32P-postlabeling assay (19), with minor modifications (16): Briefly, DNA (10-20 μg) was hydrolyzed to deoxyxynucleoside 3'-monophosphates with micrococcal nuclease and spleen phosphodiesterase [enzyme:substrate, 1:7 (w/w), 37°C, 5 h]. Adducts were enriched by selectively hydrolyzing the 3'-monophosphate of the non-added nucleotides with nuclease P1 [enzyme:substrate, 1:3 (w/w), 37°C, 45 min]. Adducts were 3'-5' labeled in the presence of T4 polynucleotide kinase and a molar excess of [γ-32P] ATP (30 μCi; 4 μM; 400 Ci/mmol). The labeled adducts were resolved by multidirectional polyethyleneimine-cellulose (PEI-cellulose) TLC. Chromatography conditions were as described previously (16), with the exception of D3 and D4 solvents, where 0.35 M ammonium hydroxide [0.8:1 (v/v)] till top of the sheet in D4, and developments in D2 and D5 were omitted. Adducts were visualized by intensifying screen-enhanced autoradiography and the radioactivity determined by Cerenkov counting. To calculate adduct levels, total nucleotides were analyzed by labeling a dilute DNA digest (2 ng) in parallel with adducts, followed by separation of labeled normal nucleotides (0.1 ng) in 1.5 M ammonium formate, pH 3.5 (20).

Adduct levels were determined by relative adduct labeling (RAL) as:

\[
\frac{c.p.m. \text{ in adducts}}{c.p.m. \text{ in total nucleotides} \times \text{dilution factor}} = \text{RAL}.
\]

Statistical methods

Data responses were analyzed as log transformations since the standard deviation increased as group means increased. Comparison of mean responses for log-transformed data were made by Dunnett's many-to-one t-test procedure.

Results

Microsomal assay

BP (10 μM) when activated with Aroclor 1254-induced micromesos in the presence of DNA resulted in the formation of two major adducts (Figure 1A): one derived from the interaction of BPDE with deoxyguanosine (dG) (adduct #1) (309 adducts/10⁷ nucleotides) and the other from further activation of 9-OH-BP (adduct #2) (34 adducts/10⁷ nucleotides) (8); no adduct spots were detected in DNA incubated with vehicle alone (data not shown). This adduct pattern was observed in all treatments in the presence or absence of 150...
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A. Control  
B. NAC  
C. BHT  
D. Curcumin  
E. Oltipraz  
F. Ellagic Acid

**Fig. 1.** Autoradiograms of $^{32}$P-postlabeling analysis of DNA reacted with microsome-generated metabolites of benzo[a]pyrene (10 μM) in the absence (A) or presence of indicated chemopreventive agents (150 μM) (B–F). BHT, butylated hydroxytoluene; and NAC, N-acetylcysteine.

**Fig. 2.** DNA adduct levels measured after microsome-mediated metabolism of benzo[a]pyrene (10 μM) in the absence or presence of indicated chemopreventive agents (150 μM). Values represent an average (± SE) of 3–6 replicates. Numbers above each bar represent percent of control values.

μM N-acetylcysteine, BHT, curcumin, oltipraz and ellagic acid (Figure 1B–F, respectively). Measurement of the adduct radioactivity revealed that with the exception of N-acetylcysteine, all test agents significantly modulated the DNA adduct formation (Figure 2). N-Acetylcysteine (150 μM) was an ineffective inhibitor of both BPDE–dG and 9-OH-BP-derived adducts having percent of control values of 105% and 103%, respectively. Curcumin significantly inhibited BPDE–dG by 57% ($P < 0.05$) while significantly enhancing the 9-OH-BP adduct up to 230% ($P < 0.05$). BHT inhibited BPDE–dG formation by 38% but it did not appear statistically significant (for two data sets $P < 0.05$, $P = 0.08$) while the 9-OH-BP adduct was significantly enhanced up to 650% ($P < 0.05$). Oltipraz and ellagic acid significantly inhibited BPDE–dG ($P < 0.05$) by 64% and 94%, respectively and 9-OH-BP adduct by 87% ($P < 0.05$) with both agents. Increasing BP concentration to 20 μM produced comparable results upon intervention with curcumin, oltipraz and ellagic acid (150 μM) (data not shown).

Lower concentrations of curcumin and BHT (10 and 50 μM) were examined to establish a dose-response relationship (Figures 3 and 4, respectively). Neither curcumin nor BHT was effective at significantly inhibiting the BPDE–dG adduct at these lower levels while both compounds enhanced 9-OH-BP adduct formation at 50 μM concentrations by 170% ($P < 0.05$) and 320% ($P < 0.05$), respectively.

**Non-microsomal assay**

BPDE (1 μM) was incubated with each of the five chemopreventive agents in the presence of DNA to determine if a non-specific reaction occurred between these agents and BPDE. One predominant adduct, identified as BPDE–dG (21), was observed in all cases. BHT, oltipraz, curcumin and N-acetyl-
Oltipraz, W-Acetylcysteine, Curcumin

Discussion

DNA adducts represent an early, detectable and critical step in the process of chemical carcinogenesis. This relationship between DNA adduct formation and carcinogenicity has been described due to the following observations: (i) adduct forming capacity and in vivo carcinogenic potency correlate for many PAHs and alkylating agents; (ii) adduct formation and in vitro cell transformation/tumor induction have a positive correlation; and (iii) adducts are generally higher in the target organs of sensitive versus resistant animal species (3). DNA adducts are also net representatives of activation and detoxification processes and can be studied both in vitro and in vivo (8). For these reasons DNA adducts are excellent biomarkers for screening potential chemopreventive agents. In this study two BP-derived DNA adducts, BPDE-dG and 9-OH-BP adduct, were used as an endpoint to examine the efficacy of five compounds and to determine the usefulness of an in vitro cell-free system as an early screening method.

Ellagic acid and oltipraz were the most efficacious compounds tested. Ellagic acid inhibited both BPDE–dG and 9-OH-BP adducts almost completely (94% and 87%, respectively). Studies have shown that ellagic acid inhibits mixed function oxidases (22), which are responsible for metabolizing PAHs to their reactive forms. Additionally, this polyphenol is known to directly bind with BPDE thereby preventing this mutagenic metabolite from interacting with DNA (23). These data indicate the inhibition of BPDE–dG by ellagic acid may be a result of both P450 inhibition and direct conjugation of BPDE. Our data confirms the likelihood of the latter mechanism since ellagic acid inhibited BPDE–dG adduct formation by 90% in the microsome-free system. The 9-OH-BP adduct has been speculated to be formed by interaction of the electrophilic species 9-OH-BP-4,5-epoxide with DNA (8). Therefore, reduction of the 9-OH-BP adduct by ellagic acid might have also resulted by direct interaction of 9-OH-BP-4,5-epoxide with ellagic acid, although ellagic acid-mediated inhibition of the activating enzymes could also contribute to the reduction of 9-OH-BP adduct formation.

Oltipraz, a 1,2-dithiole-3-thione, has been shown to inhibit tumor formation and multiplicity induced by chemically diverse carcinogens in a variety of organs including breast, colon and liver (14). However, oltipraz failed to inhibit BP induced lung tumorgenesis in A/J mice (24). The known anticarcinogenic effects of oltipraz have been correlated with induction of phase II enzymes including glutathione-S-transferase and EH (14,25). In our study, oltipraz was highly effective in inhibiting the formation of BPDE–dG and 9-OH-BP adducts (by 64% and 87%, respectively). Enzyme induction is not a plausible mechanism for this inhibition since a cell-free system was used, however, enhancement of the enzymatic activity by this agent is possible. Additionally, oltipraz was found to inhibit cytochrome P450 1A2 and 3A4 in human hepatocytes (26). However, this study did not address P450 1A1 isozymes specifically, which are responsible for metabolic activation of BP. The direct conjugation of BPDE by oltipraz can be eliminated as a likely mechanism for adduct inhibition since no inhibition of BPDE–dG binding was observed in the absence of microsomes. Therefore, the inhibition of BP–DNA adducts may be brought

Table I. Levels of BPDE-dG formed after reaction of amni benzo[a]pyrene-7,8-diol-9,10-epoxide (1 μM) with DNA in the absence or presence of various chemopreventative agents (150 μM)

<table>
<thead>
<tr>
<th>Chemopreventative agent</th>
<th>Adduct/10¹⁷ nucleotides</th>
<th>Percent of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2701±238</td>
<td>100</td>
</tr>
<tr>
<td>BHT</td>
<td>2794±408</td>
<td>103</td>
</tr>
<tr>
<td>Curcumin</td>
<td>3310±450</td>
<td>123</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>253±31</td>
<td>9</td>
</tr>
<tr>
<td>N-Acetylcysteine</td>
<td>3656±388</td>
<td>135</td>
</tr>
<tr>
<td>Oltipraz</td>
<td>2907±352</td>
<td>108</td>
</tr>
</tbody>
</table>

cysteine (150 μM) had little or no effect on the adduct level when compared with control, while ellagic acid (150 μM) inhibited adduct formation by 90% (Table I).

Fig. 3. DNA adduct levels measured after microsome-mediated metabolism of benzo[a]pyrene (10 μM) upon intervention with different doses (10, 50 and 150 μM) of curcumin. Values represent an average (± SE) of 3–5 replicates.

Fig. 4. DNA adduct levels measured after microsome-mediated metabolism of benzo[a]pyrene (10 μM) upon intervention with butylated hydroxytoluene (10, 50 and 150 μM). Values represent an average (± SE) of 3–5 replicates.
about by other plausible mechanisms including, conjugation of the electrophilic species by a metabolite of oltipraz and inhibition of P4501A1. Curcumin and BHT affected BP-DNA adduction in a similar fashion. Both agents inhibited BPDE-dG formation but the 9-OH-BP adduct was substantially enhanced (Figure 2). The enhancement of the 9-OH-BP adduct was also observed at lower concentrations (50 μM) for both curcumin (170%) and BHT (320%). A plausible mechanism for the observed inhibition of BPDE-dG concomitantly with enhancement of the 9-OH-BP adduct may involve inhibition of EH although there is no direct evidence for this mechanism. Cytochrome P4501A1 and EH convert BP to BPDE with BP-7,8-diol as an intermediate. Another electrophilic species of BP is presumably 9-OH-BP-4,5-oxide. Since this epoxide is a plausible substrate for EH detoxification, the inhibition of EH would result in an increase in observed 9-OH-BP adduct formation while BPDE-dG formation would decrease since EH is directly linked to its formation. It has also been demonstrated that BPDE is a poor substrate for microsomal EH (27,28). This hypothesis is substantiated by studies in which a decrease in BPDE-dG in concert with an increase of the 9-OH-BP adduct were observed under conditions of selective EH inhibition by 1,1,1-trichloropropane, 2,3 oxide (29). This action of inducing one adduct while inhibiting another may explain observations of tumor enhancement in one tissue type but tumor inhibition in another by BHT (12).

N-Acetylcyesteine, a synthetic aminothiol, did not influence either BPDE-dG or 9-OH-BP adduct levels in the absence or presence of microsomes. This suggests that N-acetylcyesteine does not act as an electrophilic trapping agent or P4501A1 inhibitor. The reported inhibition of BPDE-dG in BP-treated rats (30) may be related to enhancement of detoxification enzymes, altering DNA-repair processes and/or acting as a precursor for reduced glutathione (15,31).

The observations made in this study emphasize the importance of studying individual adducts as compared to total DNA binding. For example, if total binding was assessed with respect to curcumin or BHT one would determine that these compounds increase BP-DNA binding up to two-fold. In actuality, curcumin and BHT inhibited the BPDE adduct, which is believed to be the primary species in BP induced carcinogenesis, while enhancing the 9-OH-BP-derived adduct. Studying individual adducts may also provide information regarding mechanisms of chemopreventive agents which may not be made if only considering total binding.

Human industrial exposure to BP has been detected in levels of up to 4 μM although, non-occupational exposures are much lower (32), while achievable plasma levels, without significant adverse effects, of oltipraz and N-acetylcyesteine approximate 25 μM (33,34). In this study concentrations of both BP and the chemopreventive agent were higher than the expected exposure or plasma levels of these compounds while the relative ratio of the two was maintained. These relatively high concentrations of carcinogen and chemopreventive agent were chosen in order to accomplish high adduct levels which aid in studying the inhibitory effects of the chemopreventive agents.

The human capacity to bioactivate many carcinogens may be similar to that found in laboratory animals. Many human cell systems, including hepatocytes, cultured bronchus and primary lymphocytes have been shown to metabolize BP and other carcinogens, to reactive metabolites which form chromatographically identical DNA adducts to those found in animal models (35–37). Furthermore, the levels of DNA adducts found in peripheral blood lymphocytes of rats is comparable to that found in human peripheral blood lymphocytes obtained from humans after exposure to a variety of PAHs (38). However, human hepatocytes were shown to have a greater total level of carcinogen-DNA adducts when compared to rat hepatocytes exposed to the same concentration of carcinogen (36). The reactive metabolites of BP have also been shown to travel in the systemic circulation where they may exert their carcinogenic effects on distal organs (39) and (J.Arif and R.C.Gupta, submitted). These data indicate that the mechanism of DNA adduct alteration by chemopreventive agents observed in this in vitro assay may be similar to that expected to occur in humans.

The potential of this microsome-mediated system combined with 32P-postlabeling to evaluate the efficacy and mechanisms of DNA adduct-modulating chemopreventive agents has been demonstrated by its sensitivity and versatility. The high sensitivity of this test system allows the detection of DNA adducts from multiple pathways at levels as low as 1 adduct per 10^10 nucleotides (3). Although the effects of these chemopreventive agents on enzyme induction and epigenetic carcinogens can not be assessed, this assay is a valuable tool for studying the mechanistic aspects of chemopreventive agents. The addition of cytosolic enzymes to this microsomal system may provide further mechanistic information about these agents, enabling testing of diverse classes of carcinogens, in addition to PAHs. In conclusion, this assay may be useful to rapidly study the effect of potential chemopreventive agents to modulate DNA binding of diverse classes of carcinogens.

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References


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