Inhibition of Oxidative Stress in HeLa Cells by Chemopreventive Agents

Ramesh S. Bhimani, Walter Troll, Dezider Grunberger, and Krystyna Frenkel


ABSTRACT

12-O-Tetradecanoylphorbol-13-acetate (TPA)-mediated oxidative stress in HeLa cells and its inhibition were studied by fluorometric measurement of H_2O_2 and by 1H-postlabeling of the oxidized bases 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 5-hydroxymethyl-2'-deoxyuridine (HMdU). Treatment (10 nmol/cell) caused a 10-fold increase in H_2O_2 levels (0.1 nmol TPA/ml), and a 5–10-fold increase in 8-OHdG and HMdU (10 nmol TPA/ml). Naturally occurring compounds (cafeic acid phenethyl ester (CAPE), (+)-epigallocatechin gallate (EGCG), penta-O-galloyl-β-D-glucose (PGG) and sarcophytol A (Sarp A)) and the anticancer drug tamoxifen (TAM) were tested as potential chemopreventive agents. These agents dose-dependently inhibited TPA-induced H_2O_2, 8-OHdG and HMdU. The doses required for a 50% decrease in H_2O_2 were ~2.5 μM for TAM; 5 μM for CAPE, EGCG and PGG; and 75 μM for Sarp A. TAM and PGG (10 μM), EGCG (25 μM), and CAPE (50 μM) abolished TPA-mediated H_2O_2 production, even below the normal cellular levels. TAM (2.5–20 μM) decreased TPA-mediated HMdU and 8-OHdG formation 2–29 times. Maximum inhibition occurred at 20 μM TAM, which caused an ~95% decline in HMdU and 8-OHdG. CAPE was effective at 0.5–50 μM. CAPE (25 μM) decreased 8-OHdG 95% and HMdU 58%, while Sarp A (250 μM) reduced 8-OHdG by 93% and HMdU by 78%. EGCG (1–25 μM) and PGG (1–10 μM) inhibited formation of 8-OHdG and HMdU dose-dependently. However, higher doses (50 and 100 μM) decreased the efficacy of that inhibition. Of those agents tested, TAM appears to be the most and Sarp A the least effective. Our results point to these 5 compounds as being potential chemopreventive agents, which at very low doses decrease the tumor promoter-mediated oxidative processes.

INTRODUCTION

ROS, such as O_2^−, H_2O_2, and ·OHs, can be generated during aerobic cellular metabolism (1). In addition to their significant contribution to mutagenesis, carcinogenesis, and tumor promotion (reviewed in Refs. 1 and 2), ROS have been implicated in the etiology of various forms of cancer (1, 3, 4). ROS induce strand breaks in DNA and oxidative modification of DNA bases, which are implicated in the oxidative modification of DNA bases, which are implicated in the oxidative damage to cellular DNA.

In this work, we investigated several naturally occurring compounds and an anticancer drug (TAM) as potential chemopreventive agents, to establish whether they are capable of inhibiting the tumor promoter TPA-induced H_2O_2 production by HeLa cells and oxidative damage to cellular DNA.

Propolis, a honeybee-hive product, possesses anti-inflammatory, antiviral, immunostimulatory, and carcinostatic activities (19). The active component of propolis, identified as CAPE (Fig. 1), was used in this study. Sarp A (Fig. 1) is a naturally occurring nonoxic cembrane-type diterpene isolated from the soft coral Sarcophyton glaucum (20), with a potent antitumor promoting activity (20–22).

EGCG (Fig. 1), the principal polyphenolic constituent present in green tea leaves, possesses antimutagenic and anticarcinogenic properties (23–27). PGG (Fig. 1) (obtained by methanolysis of tannins isolated from the gall Schisandrae fructus) inhibits tumorigenesis in mice initiated with 7,12-dimethylbenz(a)anthracene and promoted by repeated applications of teleocidin (28, 29).

TAM (Fig. 1) is one of the most widely used drugs in the chemotherapy of breast cancer (reviewed in Refs. 30 and 31).

CAPE, Sarp A, EGCG, PGG, and TAM inhibit TPA-mediated H_2O_2 production by human neutrophils (32–34) and by differentiated human myeloid leukemia HL-60 cells (35). Sarp A, CAPE, and EGCG also suppress the TPA-induced opacification process and elevation of H_2O_2 in bovine lens (33, 36).

Similar to other cancer cells (37), HeLa cells on their own generate substantial amounts of H_2O_2 (38). They also contain measurable levels of HMdU in their DNA, which are enhanced by TAM (39). Hence, our aim was 2-fold: (a) to determine whether CAPE, Sarp A, EGCG, PGG, and TAM suppress TPA-mediated H_2O_2 and oxidized DNA base formation in HeLa cells; and (b) to establish whether these agents can inhibit oxidant formation in cells that were not treated with TPA, and thus decrease their capacity to generate H_2O_2.

MATERIALS AND METHODS

Chemicals

EGCG, PGG, and Sarp A were kind gifts from Dr. Fujiki (National Cancer Center Research Institute, Tokyo, Japan), while CAPE was synthesized as described (19). TAM, trans-TAM, dimethyl sulfoxide, horseradish peroxidase, HMdU, and catalase were purchased from Sigma Chemical Co. (St. Louis, MO). DCFdAc and DCF were obtained from Kodak (New York, NY), and dissolved in 100% ethanol. Stock solutions of TPA, TAM, CAPE, and Sarp A were prepared in dimethyl sulfoxide. EGCG was dissolved in glucose-containing BSS (137 mM NaCl, 5 mM KCl, 0.8 mM MgSO_4, 8.5 mM Na_2HPO_4, and 5 mM glucose, pH 7.4; HPLC, high pressure liquid chromatography; ACN, acetonitrile; AcO_2, acetic anhydride).
mM NaH₂PO₄, and 5 mM glucose, pH 7.4). 8-OHdG was synthesized according to published procedures (40).

Cell Culture

HeLa cells (obtained from American Type Culture Collection, Rockville, MD) were grown either in T-flasks or spinner culture bottles, using Joklik-modified minimal essential medium (GIBCO, Gaithersburg, MD) supplemented with 5% newborn calf serum (BioWhittaker, Walkersville, MD), 1% glutamine, and 1% antibiotics (GIBCO, Gaithersburg, MD). Cultures were maintained in a humidified chamber with 5% CO₂ in air and subcultured every 4–7 days using 0.25% trypsin-EDTA (GIBCO). Care was taken to use cells at the midlog growth phase for all of the experiments.

H₂O₂ Assay

To prepare standard curve, H₂O₂ was determined fluorometrically using DCFdAc that was deacetylated by treatment with NaOH just prior to use (38, 41). DCFdAc and its deacetylated product, DCFH, are both nonfluorescent. DCFH is easily oxidized to the fluorescent derivative DCF in a peroxidase-mediated reaction. This property was utilized as the basis for the determination of H₂O₂. Pretreatment with DCFdAc leads to the uptake of this lipophilic compound by cells, where it is deacetylated to DCFH by the cellular esterase (42). The DCFH is trapped inside because it is more polar than diacetate and cannot permeate the membranes. If H₂O₂ is present in the cells, it oxidizes that DCFH to fluorescent DCF utilizing cellular peroxidase as a mediator. Fig. 2 shows a schematic representation of the reactions involved.

HeLa cells in their exponential phase of growth were harvested from a spinner flask and washed twice in BSS. Cells were counted in a hemocytometer and the viability was determined by trypan blue exclusion. Cells (10⁵/ml BSS) were preincubated with sodium azide (25 mM) for 1 min and DCFdAc (10 μM) for 20 min, followed by incubation with one of the agents under study for 5 min, and then treatment with TPA (10 fmol/cell, 100 nm) for 1 h, at 37°C. The reaction was stopped by adding catalase (100 units), and the cells were lysed with sodium dodecyl sulfate (0.2%) at 37°C for 10 min. Samples were centrifuged (Rotor 6976; Beckman, Fullerton, CA) at 5000 rpm at 4°C for 10 min, and fluorescence of the supernatants determined at ex,em = 475 nm and 525 nm (spectrofluorometer model MK-2; Farrand, Valhalla, NY), using 5-nm slits. Fluorescence of standard DCF solutions was always determined prior to each experimental series to make sure that the instrument was performing properly.

Determination of Oxidized Bases in Cellular DNA

Treatment of Cells. Hela cells (10⁵) were treated with various concentrations of test agents followed by TPA (10 fmol/cell). The volume was brought up to 10 ml with BSS (final TPA concentration, 10 nmol/ml) and incubated at 37°C for 3 h with gentle shaking. After incubation, the cells were washed twice with BSS and the pellet was used for DNA isolation. The TPA control consisted of all components except the test agent, whereas the cell control lacked both TPA and the test agent.

DNA Isolation. DNA was isolated using an A.S.A.P. kit (Boehringer Mannheim, Indianapolis, IN), according to the manufacturer’s instructions. Briefly, the cells were disrupted with the lysis buffer, and incubated with heat-treated RNase at 37°C for 30 min and with proteinase K at 55°C for 30 min. The lysate was mixed with the pre-equilibrated matrix in the column and allowed to settle for 10 min. The column was washed and primed, and the DNA eluted with the elution buffer and precipitated with isopropanol. After washing with 70% ethanol and drying, the DNA was dissolved in 10 mM Tris-HCl buffer (pH 7.4), and its amount determined by assuming that 1 A₂₆₀/mg = 50 μg double-stranded DNA. This method provides a good yield of DNA with a 260/280-nm absorbance ratio of 1.7–1.9 (43).

DNA Analysis for the Presence of Oxidized Nucleosides. The DNA sample was vortexed 3 times for 30 s each at maximum speed and digested to nucleosides at 37°C for 2.5 h with DNase I in the presence of MgCl₂ (pH 7.4), followed by nuclease P1 in the presence of ZnSO₄ (pH 5.1) and alkaline phosphatase (pH 7.4), according to the published procedure (43). A 3H-post-labeling method was used for the analysis of oxidized nucleosides released after the DNA digestion, as described previously (43). Briefly, oxidized nucleosides were separated from normal nucleosides by HPLC on the octadeclisilic acid column (25 x 1-cm inner diameter, 5-μm particle size; Alltech, Beckman) in the absence of any external markers. The fractions were eluted with water for 40 min at 2 ml/min, followed by 2.5% ACN in water for another 40 min, and 5% and 10% ACN for 20 min each. Fractions that eluted after 15 min, except those containing the normal nucleosides dC, dG, dT, and dA (Table I), were combined, dried, and acetylated with Ac₂O (DuPont, Boston, MA) in the presence of the catalyst 4-dimethylaminopyridine and triethylamine at 30°C for 2 h (43, 44). The reaction was stopped by addition of methanol. The sample was dried under reduced pressure, dissolved in 20% ACN, and mixed with nonradioactive acetates of HMdU and 8-OHdG, filtered through a 0.2-μm membrane and injected into the octadecylsilic acid column. The mixture was eluted first with 10% ACN for 10 min at a flow rate of 2 ml/min, followed by an ACN-water gradient to 17% during 20 min, and held for 60 min. Elution was monitored by UV detection (model 164; Beckman, Fullerton, CA) and radioactive liquid scintillation counting (Tecl Analytic, Elk Grove Village, IL). Retention times of the acetates are listed in Table I.

Quantitation of Oxidized Nucleosides. The concentrations of normal nucleosides eluting from the HPLC column were calculated by preparing standard calibration curves. Quantitation of oxidized nucleosides was based on HPLC separation of their acetates and was carried out by using the following formula (43): pmol nucleoside = net cpm of nucleoside acetate/(0.3 x conversion coefficient x no. of acetyl groups). In this formula, 0.3 is the efficiency of liquid scintillation counting (30%), and the conversion coefficient depends on the specific activity of Ac₂O (either 22 or 11.1 when Ac₂O is diluted with nonradioactive acetic anhydride 1:5 or 1:10, respectively). The results are reported as oxidized bases/10⁴ normal bases in DNA, unless otherwise stated.

Table I HPLC separation of nucleosides and their acetates

<table>
<thead>
<tr>
<th>Nucleosides</th>
<th>Rₙ (min)</th>
<th>Acetates</th>
<th>Rₙ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMdU</td>
<td>52</td>
<td>HMdU/Ac₂</td>
<td>75</td>
</tr>
<tr>
<td>8-OHdG</td>
<td>88</td>
<td>8-OHdG/Ac₂</td>
<td>53</td>
</tr>
<tr>
<td>dC</td>
<td>45</td>
<td>dC</td>
<td></td>
</tr>
<tr>
<td>dG</td>
<td>68</td>
<td>dG</td>
<td></td>
</tr>
<tr>
<td>dT</td>
<td>74</td>
<td>dT</td>
<td></td>
</tr>
<tr>
<td>dA</td>
<td>99</td>
<td>dA</td>
<td></td>
</tr>
</tbody>
</table>

_Rₙ_ retention time; Ac₂O, triacetate.
TPA-induced H₂O₂ Production by HeLa Cells. HeLa cells exhibited a dose-dependent increase in H₂O₂ production when treated with TPA (Fig. 3), reaching maximum at 10 fmol TPA/cell (0.1 nmol/ml), after which it plateaued. Since the most effective TPA dose that gave the maximum H₂O₂ production (6-7-fold over the untreated control) was 10 fmol/cell, this dose was used in all the experiments.

Statistics

Results are expressed as means ± SE obtained from 2 or 3 experiments. Statistical significance was evaluated using the Student’s t test.

RESULTS

Table 2 Effect of test agents on formation of 8-OHdG in the DNA of HeLa cells (% change)

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>TAM</th>
<th>CAPE</th>
<th>EGCG</th>
<th>PGG</th>
<th>Sarp A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>—</td>
<td>63.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2.5</td>
<td>74.6±5.5</td>
<td>3.6</td>
<td>46.1±14.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5.0</td>
<td>—</td>
<td>75.0</td>
<td>62.5±19.7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10.0</td>
<td>84.2±11.0</td>
<td>—</td>
<td>76.5±12.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>20.0</td>
<td>943±2.5c</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>25.0</td>
<td>—</td>
<td>95.1</td>
<td>80.0±12.0</td>
<td>80.5±10.5</td>
<td>—</td>
</tr>
<tr>
<td>50.0</td>
<td>—</td>
<td>97.5</td>
<td>12.5±33.7</td>
<td>42.8±33.7</td>
<td>—</td>
</tr>
<tr>
<td>100.0</td>
<td>—</td>
<td>66.5±83.5†</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>125.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>83.5±9.5</td>
</tr>
<tr>
<td>250.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>93.0±3.0</td>
</tr>
</tbody>
</table>

* Values are from 1 to 3 independent experiments. Percentages were calculated from results of paired experiments (TPA ± test agent) and are presented as percent decrease from the levels induced by TPA, except where an increase is indicated by †.

†, not determined.

Table 3 Effect of test agents on formation of HMdU in the DNA of HeLa cells (% change)

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>TAM</th>
<th>CAPE</th>
<th>EGCG</th>
<th>PGG</th>
<th>Sarp A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>—</td>
<td>—</td>
<td>22.0</td>
<td>46.2±25.2</td>
<td>—</td>
</tr>
<tr>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>28.0</td>
<td>48.0±20.7</td>
<td>—</td>
</tr>
<tr>
<td>2.5</td>
<td>65.0±12.0</td>
<td>—</td>
<td>76.7±13.5</td>
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<td>—</td>
</tr>
<tr>
<td>5.0</td>
<td>—</td>
<td>80.0±9.2</td>
<td>—</td>
<td>80.3±1.8</td>
<td>—</td>
</tr>
<tr>
<td>10.0</td>
<td>96.0±0.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>20.0</td>
<td>—</td>
<td>58.0</td>
<td>89.0±0.7</td>
<td>55.2±33.8</td>
<td>—</td>
</tr>
<tr>
<td>25.0</td>
<td>—</td>
<td>6.2</td>
<td>61.0±11.2</td>
<td>87.1±5.9</td>
<td>—</td>
</tr>
<tr>
<td>50.0</td>
<td>—</td>
<td>41.0</td>
<td>53.9</td>
<td>57.2±36.7</td>
<td>—</td>
</tr>
<tr>
<td>100.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>78.0±16.0</td>
</tr>
<tr>
<td>250.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</tr>
</tbody>
</table>

* Values are from 1 to 3 independent experiments. Percentages were calculated from the results of paired experiments (TPA ± test agent) and are presented as percent decrease from the levels indicated by TAM except where an increase is indicated by †.

†, not determined.

Effect of Chemopreventive Agents on TPA-mediated H₂O₂ Production. All of the compounds studied (EGCG, PGG, Sarp A, CAPE, and TAM) suppressed in a dose-dependent manner the TPA-mediated H₂O₂ formation by HeLa cells (Fig. 4, a-e). The doses required to inhibit 50% of the H₂O₂ generated by the action of TPA were about 2.5 μM for TAM; 5 μM for CAPE, EGCG, and PGG; and 75 μM for Sarp A. Higher concentrations of TAM and PGG (10 μM), EGCG (25 μM), and CAPE (50 μM) abolished TPA-mediated H₂O₂ formation, even below the normal cellular level (produced in the absence of TPA and the test agent). The order of effectiveness of these agents, with respect to their inhibition of TPA-mediated H₂O₂ formation, was TAM > EGCG > PGG and CAPE > Sarp A.

TPA-induced Formation of Oxidized Bases and Inhibitory Effects of Chemopreventive Agents. Analysis of the oxidized DNA base derivatives isolated from the DNA of HeLa cells showed that at a dose of 10 fmol/cell (10 nmol/ml), TPA induced HMdU and 8-OHdG formation at the levels of 7.25 ± 2.2 and 5.6 ± 2.5/10⁴ normal bases, which were 8- and 11-fold higher than the untreated controls (0.9 ± 0.4 and 0.5 ± 0.1/10⁴ bases, respectively). All of the test agents investigated decreased the TPA-induced formation of HMdU and 8-OHdG in the HeLa cell DNA. At the doses of 2.5-20 μM, TAM suppressed HMdU 3- to 24-fold and 8-OHdG 4- to 21-fold. Maximum inhibition was achieved at 20 μM TAM, a concentration at...
We found that TPA treatment increases the formation of H$_2$O$_2$, HMdU, and 8-OHdG over the respective untreated controls. TPA is known to cause a decrease in the activities of antioxidant enzymes, such as superoxide dismutase and catalase (47, 48), in mouse skin. So it is likely that this would also occur in HeLa cells. However, the residual enzyme activities could probably still lower the levels of TPA-induced O$_2^-$ and H$_2$O$_2$, respectively, and to some extent, protect the cells from the action of these oxidants. The oxidative DNA damage incurred due to the ROS generated by the TPA-mediated processes can also be repaired by DNA glycosylases and endonucleases, which recognize oxidized bases in DNA (8–13). Therefore, the measured levels of oxidized bases in DNA reflect a dynamic equilibrium between their formation and removal.

The elevated levels of H$_2$O$_2$ produced in response to TPA treatment (6- to 7-fold over control) cannot be implicated solely in the formation of oxidized DNA bases. This is because H$_2$O$_2$ also can be utilized in oxidation of other cellular macromolecules, and some H$_2$O$_2$ may be decomposed to molecular oxygen and water by cellular catalase (18). However, neither O$_2^-$ nor H$_2$O$_2$ under physiological conditions appears to produce DNA strand breakage or oxidative modification of DNA bases (49). It is known that high H$_2$O$_2$ doses are required to cause a mutagenic event in mammalian cells (49). This may be due in part to the degradation of the externally applied H$_2$O$_2$ by transition metal ions present in the medium. The remaining H$_2$O$_2$ readily crosses cellular membranes and, in the presence of liganded Fe and Cu, -OHs can be generated that damage the cellular macromolecules, including DNA, present in close proximity to the -OH production (49).

TAM inhibited TPA-mediated H$_2$O$_2$ production at the low dose of 1 nmol/ml, while at 10 nmol/ml H$_2$O$_2$ was decreased even below that of an untreated control (no TPA, no TAM). These results suggest that TAM can inhibit even the background cellular H$_2$O$_2$ production by HeLa cells. TAM exerts its antiproliferative effects on both the estrogen receptor positive and negative breast cancer cell lines (30, 31).

Recently, TAM was found to down-regulate c-erb B-2 and c-myc oncogenes (50). Thus, the suppression of breast cancer by TAM may involve multiple mechanisms, in addition to the competitive interactions with the estrogen receptors, which cause a decrease in the mitotic function of estrogen (30, 31). Malins and Hainanot (51) showed that DNA of invasive ductal carcinomas from 4 women contained up to 9-fold higher levels of the oxidized purines, and the open ring products derived from them, than the surrounding tissue. The observation that TAM inhibits H$_2$O$_2$ production and the consequent formation of HMdU and 8-OHdG in human cancer cells provides a novel possible explanation for its antitumor activity. This mechanism would involve preventing oxidant formation and the subsequent oxidation of DNA bases induced by tumor promoters, including those present in the diet. The mechanism by which TAM suppresses H$_2$O$_2$ formation is not known as yet; however, since TAM is a PKC inhibitor, this would be the likely but might not be the only step. It could be any of the enzymatic steps involved in the activation of reduced nicotinamide-adenine dinucleotide phosphate oxidase and reduced nicotinamide-adenine dinucleotide phosphate regeneration.

In addition to its carcinostatic and antimutagenic activities (23–27), EGCG prevents formation of ionizing radiation-induced lipid peroxides in the liver and significantly prolongs the life span of the mice after lethal whole body X-irradiation (52). As we found, EGCG also inhibits the tumor promoter TPA-mediated H$_2$O$_2$ production and the formation of oxidized DNA bases in HeLa cells. This may be due to the capacity of EGCG to suppress the binding of the tumor promoter to the receptors, since EGCG was shown to inhibit the activation of protein kinase C by teleocidin B (23). The EGCG concentration (25 μM) that suppressed TPA-mediated H$_2$O$_2$ production even below normal cellular levels also caused a maximum decline in the formation of DNA bases.
oxidized bases. However, increasing the EGCG dose to 50 and 100 nmol/ml decreased its efficacy for inhibiting H2O2 and oxidized DNA base formation (Fig. 4, Tables 2 and 3). That this may be a more general property of tannins is shown by our results in which PGG dose-dependently inhibited TPA-induced formation of H2O2 and 8-OHdG in HeLa cells at 1–10 nmol/ml but nullified that inhibition at 50 nmol/ml. Hence, it appears that at low doses tannins suppress tumor promoter-mediated ROS production and oxidative DNA damage. Whereas higher doses could be involved in secondary reactions that may additionally produce some ROS (i.e., by redox cycling) or lead to generation of oxidants by other cellular processes.

Much higher concentrations of Sarp A were required to suppress all of the oxidative events. However, this may be due to its low solubility in the aqueous medium, since it is very active in the inhibition of tumor promotion (20–22), ROS generation, and oxidized DNA base formation at very low doses (6.5 nmol) when topically applied to mouse skin (53). Tumor promotion and ROS generation are often associated with the activation of protein kinase C and/or ODC induction. However, in the case of Sarp A, its antitumor promoting effects are not mediated via these pathways (54). Since Sarp A binds to various proteins, such as albumin, with different affinities, it may be possible that it enters the cells, binds to various proteins, and then inhibits the pathway(s) necessary for tumor promotion, most notably gene expression (54). Sarp A may also counteract the TPA-induced decline in the level of antioxidant enzymes and consequently inhibit the formation of oxidized bases, or inhibit an enzymatic step necessary for the generation of ROS.

CAPE inhibited TPA-mediated H2O2 production dose-dependently and decreased it slightly below the normal control level at 50 nmol/ml. The maximum decrease in formation of oxidized bases occurred at 25 nmol/ml CAPE. Since in vivo 6.5 nmol CAPE suppressed TPA-induced HMDU and 8-OHdG formation below the control levels by 20% and 72%, respectively (33), the low solubility in the aqueous medium may have decreased its inhibitory efficacy in TPA-treated HeLa cells. In summary, TAM and PGG (10 μM), EGCG (25 μM), and CAPE (50 μM) suppressed TPA-mediated H2O2 production even below the normal cellular levels. Some of these agents caused a similar decline in the oxidized DNA bases. In control cells (no TPA), 8-OHdG and HMDU were present at 0.5 ± 0.1 and 0.9 ± 0.4/104 normal bases, while TPA treatment increased them to 5.6 ± 2.5 (8-OHdG) and 7.25 ± 2.2 (HMDU), respectively. After treatment with TAM (20 μM) + TPA, they declined to 0.31 (8-OHdG) and 0.29 (HMDU)/104 normal bases. The CAPE (50 μM) + TPA treatment decreased the 8-OHdG level to 0.14, while Sarp A (250 μM) decreased it to 0.39/104 bases. Although at times substantial, the decreases below the control values in the levels of H2O2 and oxidized DNA bases were not statistically different from those of the controls. Suppression of TPA-mediated formation of H2O2, 8-OHdG, and HMDU by CAPE, TAM, EGCG, and Sarp A also were observed in vivo in SENCAR mice pretreated with those agents at doses equimolar to those of TPA (33, 53, 55). Cumulatively, these results point to the significance of H2O2 as being one of the important ROS intermediates necessary for oxidative DNA damage.

With so many chemical carcinogens present in our day-to-day life, it is very difficult to escape from these toxicants. Most of the anticancer chemotherapeutic drugs have severe side effects and, therefore, cannot be used in a chemopreventive regimen. Thus, it becomes important to establish whether we can incorporate certain anticarcinogenic compounds into our regular diet to prevent the occurrence of those cancers. Research on various food components and naturally occurring compounds has opened a new approach towards chemoprevention (56). EGCG, PGG, CAPE, and Sarp A hold the promise of being useful chemopreventive agents once their toxicity to humans is proven to be negligible, especially since they can be so effective at low doses.

REFERENCES
PREVENTION OF OXIDATIVE DNA DAMAGE IN HeLa CELLS


Inhibition of Oxidative Stress in HeLa Cells by Chemopreventive Agents

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