Selective Effects of Selenium Selenite on 7,12-Dimethylbenz(a)anthracene-DNA Binding in Fetal Mouse Cell Cultures

John A. Milner, Margaret A. Pigott, and Anthony Dipple

ABSTRACT

Sodium selenite inhibits the binding of 7,12-dimethylbenz(a)anthracene (DMBA) to DNA in tertiary cultures of fetal mouse cells in a rather selective fashion. Inhibition can be demonstrated at 6 but not at 3 h after DMBA addition to the cells. Inhibition is seen after treatment of the cells with DMBA concentrations of 0.05 or 0.1 µg/ml but not after treatment at 0.01 µg/ml. Furthermore, the inhibition seen with up to 1 µg selenium/ml is selective in that products from the anti bay region dihydrodiol epoxide metabolite (where the epoxide oxygen is trans to the 4-hydroxy group) are suppressed while those from the syn-dihydrodiol-epoxide (where the epoxide oxygen is cis to the 4-hydroxy group) are not affected. In the absence of selenite, it was found that syn-dihydrodiol epoxide-DNA adducts are formed in a roughly linear fashion with time over a range of DMBA concentration. In contrast, the capacity of the cells to generate anti-dihydrodiol-epoxide adducts in a 3-h interval is saturated at concentrations of DMBA above 0.025 µg/ml and after the initial 3-h period the cells generate these adducts at up to a 6-fold greater rate than during the initial 3 h. This increase in rate of formation of anti-dihydrodiol-epoxide products is inhibited by actinomycin D and appears to be a consequence of DMBA inducing an enzyme activity which selectively generates the anti-dihydrodiol-epoxide and not the syn-dihydrodiol-epoxide. The selective action of sodium selenite in inhibiting only anti-dihydrodiol-epoxide product formation and doing this only at certain times and at certain doses of DMBA is a result of the fact that it inhibits the induction process. Once induction has occurred, sodium selenite is no longer inhibitory of DMBA-DNA binding. The chemopreventive action of selenium in chemical carcinogenesis could be partially attributable to effects such as those described herein on carcinogen-DNA binding. It is also possible, however, that the chemopreventive actions of selenium might be attributable to effects on the expression of genes other than those involved in carcinogen metabolism.

INTRODUCTION

Epidemiological studies indicate that populations residing in geographic regions where the selenium content of the soil is low exhibit higher incidences of breast, colonic, rectal, and other cancers than do the general population (1-3). In addition, an inverse correlation between mortality from several forms of cancer and apparent dietary intake of selenium has been reported (4). These observations suggest that selenium may be a naturally occurring anticarcinogenic agent and have prompted numerous experimental investigations which generally have been supportive of this hypothesis. Thus administration of selenium in the diet or drinking water to animals exposed to a wide variety of chemical carcinogens has resulted in reduced tumor incidences and/or tumor number (5-14). While most of the chemical carcinogens examined have been of the type requiring metabolic activation, selenium also inhibits the action of a directly acting carcinogen in some instances (15) but not in all (16). Selenium can be inhibitory of carcinogenesis when administered either during the initiation phase or during the promotion phase of carcinogenesis (7, 14) but the most pronounced effects are observed when selenium is given for prolonged periods of time covering both of these phases (7).

In attempts to understand the mechanisms through which selenium acts to inhibit carcinogenesis, several investigations of the effect of selenium on carcinogen metabolism (17-19), on carcinogen-DNA binding (18, 20, 21), or on macromolecular events which follow this in the development of cancer (15). One of the carcinogens which has been used extensively in examining the chemopreventive action of selenium is DMBA (7, 9, 12-15). Selenium supplementation (2-4 µg/g) of diet or drinking water has resulted in substantial inhibition of DMBA-induced mammary carcinogenesis in both rats and mice (7-9, 12, 14, 15). Dietary selenium has been shown to inhibit DMBA-induced skin tumor formation also (13).

Extensive studies of DNA-bound DMBA from mammalian cells and tissues (24-32) have indicated that the metabolic activation pathway for DMBA in these systems primarily involves the bay-region dihydrodiol-epoxide route originally postulated by Jerina and Daly (33). Recently Sawicki et al. (32) have shown that both syn- and anti-bay-region dihydrodiol-epoxides (where the epoxide oxygen and 4-hydroxy group are cis and trans to one another, respectively) are primary contributors to the binding of DMBA to DNA in fetal mouse cells in culture and these same adducts are formed when DMBA is bound to DNA in mouse skin (25, 34). It is also known that the anti-dihydrodiol-epoxide reacts to similar extents with both deoxyguanosine and deoxyadenosine residues in DNA while the syn-dihydrodiol-epoxide exhibits a marked preference for reaction with deoxyadenosine residues (27, 28). These recent developments have enabled us to examine the effects which selenium has on DMBA-DNA adduct formation in a model system of fetal mouse cells in culture. After following...
both overall binding to DNA and the amounts of individual adducts formed in the presence of various quantities or forms of selenium, we now report that selenium can have a profound effect on the metabolic activation of DMBA for DNA binding.

MATERIALS AND METHODS

Chemicals. Sodium selenite, seleno- DL-methionine, and actinomycin D were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium selenide was purchased from Alfa Products (Danvers, MA). Selenodiglutathione was synthesized by the method of Vernie et al. (23). All selenium compounds were prepared in glass distilled water and filter sterilized prior to use. Concentrations of selenium in solutions were verified by atomic absorption spectrometry.

{[G-3H]}DMBA (50 and 36 Ci/mmol) was purchased from Amersham/Searle Corp. (Arlington Heights, IL) and was routinely purified on silicic acid as described previously (29).

Cell Culture and Treatment. NIH Swiss fetal mouse cells were prepared as described previously (26). Cells were grown and passaged twice in 850-cm² roller bottles in Eagle’s minimal essential medium supplemented with 10% fetal bovine serum and 4 mM L-glutamine prior to storage in liquid nitrogen as secondary cultures. The cells were subsequently plated in 150-cm² flasks. Culture medium was changed on the first day after seeding. On the third day selenium was added to the cultures and they were treated with 0.001 vol of {[H]}DMBA in dimethyl sulfoxide. Cells were harvested by trypsinization at the desired times for each experiment, washed with phosphate-buffered saline, and pelleted. For the metabolism study, tertiary cultures were set up by plating 2 × 10⁶ cells/T-25 flask in 5 ml medium. Aliquots of medium were monitored for metabolism of {[H]}DMBA by thin-layer chromatography as described earlier (29).

Isolation and Analysis of DNA. DNA was isolated from the cell pellets by isopycnic centrifugation in cesium chloride (35) either immediately or after storage at −60°C. Cesium chloride was removed by dialysis against 0.01 M Tris-0.002 M NaCl buffer (pH 7.5). DNA solutions containing up to 0.2 mg DNA were enzymically hydrolyzed to deoxyribonucleoside mixtures by incubation at 37°C for 1 h with 0.1 vol 0.01 M MgCI₂ in 0.01 M Tris-HCl buffer (pH 7), and 0.1 ml bovine pancreatic DNase I (Worthington Biochemical Corp., Freehold, NJ) solution (1 mg/ml in 0.01 M Tris-HCl buffer, pH 7). This was followed by addition of an equal vol of 0.2 M Tris-HCl buffer (pH 9) and 0.1 ml of snake venom phosphodiesterase (from Crotalus atrox venom; Sigma) solution (1 unit/ml in 0.01 M Tris-HCl, pH 7) and incubation for a further 48 h. Bacterial alkaline phosphomonoesterase (Sigma) solution (0.1 ml; 25 units/ml in 0.01 M Tris-HCl, pH 7) was then added and incubation was continued for a further 24 h. The DNA hydrolysates were loaded onto Sep-Pak C₁₈ cartridges (Waters Associates, Milford, MA) and these were eluted with water (5 ml) and 40% methanol (eight 1-ml fractions) to remove buffer salts, unmodified nucleosides, and any incompletely digested DNA, and then with methanol (one 0.4-ml, one 1.8-ml, and two 0.4-ml fractions) to elute DMBA-deoxyribonucleoside adducts which were usually found entirely within the 1.8-ml fraction. These adducts were then analyzed by high-pressure liquid chromatography on an Altex Ultrasphere octadecyl silica column with a gradient from 45–65% methanol in water (32).

RESULTS

To determine whether selenium had any effect on the overall metabolism of DMBA, the time-dependent loss of DMBA from the culture medium of tertiary cultures of fetal mouse cells was examined (Chart 1). Data were collected for five different concentrations of each of four selenium compounds. With the exception of the data obtained for 0.4, 0.6, 0.8, and 1.0 µg selenium/ml from selenodiglutathione (Chart 1, ---), the data obtained were indistinguishable from those obtained in the absence of selenium (Chart 1, ——) indicating that the rate of overall metabolism of DMBA was not particularly sensitive to selenium. At the selenodiglutathione levels specified above, no dose response was evident. During the first 2 h, metabolism of DMBA was comparable to that of the control cultures but thereafter the rate of metabolism was dramatically reduced. In these selenodiglutathione-treated cultures, some detachment of cells from the culture flasks was apparent, indicating that this agent is cytotoxic at these higher levels.

More subtle effects of selenium on the metabolic activation of DMBA were sought through examination of DMBA-DNA binding. Table 1 illustrates that in tertiary fetal mouse cell cultures 1 µg selenium/ml of culture medium exhibited no demonstrable effect on total DMBA-DNA binding when it was administered in the form of selenomethionine or sodium selenide. However, when administered as sodium selenite, there appeared to be some inhibition of DMBA binding to DNA over a 6-h time interval but curiously no effect on the binding which occurred in 3 h. Again selenodiglutathione exerted by far the most profound effects and DMBA-DNA binding was drastically reduced at both time intervals examined. Since we have previously partially characterized the major DMBA-DNA adducts formed in mouse cells (27, 32), each of the DNA samples obtained was subjected to enzymic hydrolysis and the amounts of individual adducts present were quantified after separation by high-pressure liquid chromatography. These analyses revealed two major points of interest: (a) comparison of the control data for a 3- and 6-h exposure to DMBA indicated that by 6 h all three major adducts were present in roughly similar amounts while at 3 h the adduct derived from a syn-dihydridiol-epoxide predominated over the two adducts derived from an anti-dihydridiol-epoxide; and (b) the amount of the selenite and selenodiglutathione data at 6 h, where inhibition of the binding reaction was apparent, suggested that the for-
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The data are the averages of duplicate samples to 2 significant figures except for control which is the average of quadruplicate samples. In each column, entries not sharing a common superscript are significantly different (P < 0.05) according to Duncan's new multiple range test. Selenium compounds in phosphate-buffered saline were added to groups of four 150-cm² flasks (seeded originally with 7 x 10⁶ cells) to yield selenium concentrations of 1 µg/ml. After 1 h, these cultures together with eight cultures treated with saline only were exposed to [³H]DMBA at 0.1 µg/ml. DNA was isolated from individual flasks, one-half of which were harvested at 3 h and one-half at 6 h after exposure to DMBA and analysis was as previously described (27, 32). The three major adducts formed (see Chart 3) have been identified in the sequence of their elution in Chart 3 as an anti-3,4-dihydrodiol-1,2-epoxide-deoxyguanosine (anti-dGuo), a syn-3,4-dihydrodiol-1,2-epoxide-deoxyadenosine (syn-dAdo), and an anti-3,4-dihydrodiol-1,2-epoxide-deoxyadenosine (anti-dAdo) adduct respectively (27, 28, 32).

Table 1

<table>
<thead>
<tr>
<th>Time (min) between selenium addition and DMBA addition</th>
<th>Adducts (µmol/mol DNA-P at 3 h)</th>
<th>Adducts (µmol/mol DNA-P at 6 h)</th>
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<tr>
<td></td>
<td>Total anti-dGuo anti-dAdo</td>
<td>Total anti-dGuo anti-dAdo</td>
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<td></td>
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<tr>
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Table 2

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<th>Time (min) between selenium addition and DMBA addition</th>
<th>Adducts (µmol/mol DNA-P at 3 h)</th>
<th>Adducts (µmol/mol DNA-P at 6 h)</th>
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<tr>
<td></td>
<td>Total anti-dGuo anti-dAdo</td>
<td>Total anti-dGuo anti-dAdo</td>
</tr>
<tr>
<td>No selenium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
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<td>4.8ᵃ 0.6ᵇ 1.7ᵇ</td>
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<td>5.0ᵇ 0.6ᵇ 1.7ᵇ</td>
<td>3.6ᵇ 0.6ᵇ 1.7ᵇ</td>
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mation of the anti-dihydrodiol-epoxide-DNA adducts was reduced to a greater extent than was the formation of the syn-dihydrodiol-epoxide-DNA adducts.

Since selenium appeared to be reducing the formation of the anti-dihydrodiol-epoxide to a larger extent than for syn-dihydrodiol-epoxide, another series of experiments was undertaken to explore these effects on DMBA-DNA binding in greater detail. In these experiments, sodium selenite was used as the selenium source because some effect on binding had been noted (Table 1) while no signs of toxicity to the cultures were apparent. The time of exposure of the cultures to sodium selenite prior to the incubation with the radiolabeled carcinogen did not appear to be of major importance in determining the inhibitory effect on DMBA-DNA binding in tertiary fetal mouse cultures (Table 2). For a 3-h incubation with DMBA, selenite again showed no effect on total binding, irrespective of whether it was added at the same time as the carcinogen or at 30 or 60 min before the carcinogen.

Again selenite was inhibitory in the 6-h incubations with DMBA. Product analysis showed that at the 3-h time point where no effect on overall binding was seen no changes in individual products were detected. Inhibition of overall binding was clearly apparent at 6 h and as noted earlier this principally affected the levels of the anti-dihydrodiol-epoxide-DNA adducts. Thus there was no significant difference between the amounts of syn-dihydrodiol-epoxide-deoxyadenosine products recovered in the four 6-h experiments in Table 2, but the amounts of anti-dihydrodiol-epoxide adducts recovered decreased with the lowered overall binding to DNA. Again the preponderance of the syn-dihydrodiol-epoxide adduct in the 3-h controls differed from the roughly similar amounts of all three major adducts found in the 6-h controls.

With a fixed time of preincubation with sodium selenite of 1 h, the effects of different concentrations of selenium on DMBA-DNA binding were then investigated. As found in the other studies, the binding of DMBA to DNA in a 3-h exposure was essentially unaffected by the presence of selenium in the form of sodium selenite (Chart 2). Binding over a 6-h interval was reduced significantly in the presence of selenite, however, and the inhibitory effect increased with increasing doses of selenium. As previously observed, product analyses again showed that during a 6-h exposure to DMBA, the formation of the anti-dihydrodiol-epoxide adducts was more sensitive to the inhibitory effects of selenite than was the formation of the syn-dihydrodiol-epoxide adducts. This is illustrated in Chart 3 where one of the duplicate chromatographic analyses for each selenium dose is presented. It is clear that the 0.5- and 1.0-µg/ml doses of selenium did not notably affect the yield of the syn-dihydrodiol-epoxide adducts (Chart 3, A, D, F, and G) while the adducts from the anti-dihydrodiol-epoxide (Chart 3, B, C, and E) were substantially reduced. The highest dose of selenium, 2.0 µg/ml, inhibited...
the formation of all of the adducts. This effect is similar to that seen earlier (Table 1) with selenodiglutathione and as in that case some signs of toxicity were present in the cultures exposed to this high concentration of sodium selenite. The complete analyses for the three major adducts are summarized in Chart 4. This summary indicates that the highest dose of selenium exerted only a minimal effect on the formation of any of the three adducts in the first 3 h but that this dose of selenium led to a total shutdown of any further DNA binding of DMBA beyond 3 h. The rest of the data highlights an important difference between the kinetics of formation of the adducts derived from the syn-dihydrodiol-epoxide and those formed from the anti-isomer. For the anti-isomer, these data show that between 3 and 6 h adduct formation occurs at a much greater rate than during the first 3-h period and indicate that the carcinogen DMBA induces or activates the enzyme system responsible for formation of the anti-dihydrodiol-epoxide. The inhibitory effects of selenite arise from its partial inhibition of this rate increase either through inhibition of the induction or inhibition of the induced enzyme. Thus for the anti-dihydrodiol-epoxide, 1 µg selenium/ml led to 55–56% inhibition of adduct formation at 6 h relative to the controls. In contrast, no notable effect of selenite on the syn-dihydrodiol-epoxide adduct was observed and there was no suggestion of an activation or induction process occurring for this isomer.

A more detailed investigation of the time dependence of adduct formation from either diastereomeric dihydrodiol-epoxide in the absence of selenium confirmed the increased rate of formation of products from the anti-diastereomer after the first 3-h period and the absence of such a phenomenon for the syn-diastereomer in these tertiary fetal mouse cell cultures (Chart 5). This induction or activation pathway is clearly responsible for the remarkable difference in product distributions found in the analyses of DNA samples isolated after 3- or 6-h incubations of the cells with DMBA.

In order to distinguish between the possibilities of induction and activation in the formation of the anti-dihydrodiol-epoxide adducts, the effect of an inhibitor of RNA synthesis, actinomycin D, on the formation of these adducts was investigated. Since the effects of the induction or activation were only apparent after a 6-h exposure to DMBA, all measurements were collected using this time interval. It is clear from the data in Table 3 that exposure to the tertiary cultures to actinomycin D resulted in a more profound inhibition of DMBA binding to DNA than did exposure of the cultures to sodium selenite. Moreover as for the selenite inhibition, actinomycin D was selective in its inhibitory effects and a much more marked inhibition of the formation of anti-dihydrodiol-epoxide adducts than of syn-dihydrodiol-epoxide adducts was observed. This indicated that inhibition of RNA synthesis by actinomycin D led to inhibition of the formation of anti-dihydrodiol-epoxide adducts. Other data which indicated that selenite was affecting an enzyme system induced by DMBA were obtained from examination of the effects of selenium on DMBA-DNA binding at various concentrations of DMBA (Chart 6). As reported above, selenium in the form of sodium selenite

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**Chart 2.** Effect of increasing doses of selenium administered as sodium selenite on the overall binding of [3H]DMBA (0.1 µg/ml) to DNA in tertiary cultures of fetal mouse cells (originally seeded at 8 x 10⁶ cells/150-cm² flask). Points, averages of duplicate determinations for selenium exposures and of quadruplicate determinations for control cultures. DNA was isolated at 3 (■) and 6 h (○) after exposure to DMBA.

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**Chart 3.** Effect of sodium selenite on individual DMBA-deoxyribonucleoside adducts separated by high pressure liquid chromatography on an Ultrasphere octadecylsine column. Previous studies (27, 28, 32) indicate that the individual adducts can be tentatively identified as anti-3,4-dihydrodiol-1,2-epoxide-deoxyguanosine (B and C) and -deoxyadenosine (E) adducts and syn-3,4-dihydrodiol-1,2-epoxide-deoxycytidylate (A) and deoxyadenosine (D, F, and G) adducts. **(Note that several adducts can arise from the reaction of one diastereomeric diol-epoxide with one nucleoside residue because cis or trans opening of the epoxide ring can occur and products from the (+)- and (-)-enantiomers of diol-epoxide would be separable diastereomers.)** Each panel represents one of the duplicate (quadruplicate in the case of the control) analyses of the DNA samples exposed to [3H]DMBA (0.1 µg/ml) for 6 h in the presence of various amounts of selenium in the form of sodium selenite.
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Chart 4. Effect of various concentrations of selenium in the form of sodium selenite on the yields of the three major DMBA-deoxyribonucleoside adducts (i.e., the anti-dihydriodiol-epoxide-deoxyguanosine (anti-dGuo) and -deoxyadenosine (anti-dAdo) adducts and the syn-dihydriodiol-epoxide-deoxyadenosine (syn dAdo) adduct) formed at 3 and 6 h in the experiment described in Chart 2. Points, averages of duplicate analyses (quadruplicate for the controls). O no selenium; O 0.5 ng selenium/ml; A, 1.0 ng selenium/ml; A, 2.0 ng selenium/ml.

Chart 5. Differences in the time dependence of adduct formation from syn- and anti-dihydriodiol-epoxide metabolites of DMBA in tertiary cultures of fetal mouse cells (seeded at 8 x 10^6 cells/150-cm² flask). Cells were exposed to pHJDMBA (0.1 μg/ml) and duplicate samples were isolated at the times indicated. DNA was isolated and analyzed (as shown in Chart 3). Points, average of duplicate samples. anti-dGuo, anti-dihydriodiol-epoxide-deoxyguanosine; anti-dAdo, anti-dihydriodiol-epoxide-deoxyadenosine; syn-dAdo, syn-dihydriodiol-epoxide-deoxyadenosine.

inhibited the binding of DMBA (given at 0.1 μg/ml) and again, the anti-dihydriodiol-epoxide-deoxyguanosine and -deoxyadenosine adducts were reduced (from 4.0 and 2.9 μmol/mol DNA-P in the control cultures to 2.1 and 1.6 μmol/mol DNA-P respectively in the selenium-exposed cultures), while the syn-dihydriodiol-epoxide-deoxyadenosine adduct was hardly affected (2.4 and 2.1 μmol/mol DNA-P in the control and selenium-exposed cultures respectively) by the selenium exposure. However, at the lowest concentration of DMBA examined (0.01 μg/ml [this did not lead to a notable induction of enzyme forming the anti-dihydriodiol-epoxide (see data in Chart 8)], no inhibitory effects of selenite were detected in the levels of total binding of DMBA to DNA (Chart 6) or in the levels of the individual major adducts formed.

To determine the kinetics of adduct formation at various concentrations of DMBA, the experiment summarized in Chart 7 was undertaken. This experiment revealed several points of interest. To begin with examination of the amounts of individual adducts formed in the initial 3 h of incubation indicated that while the amounts of the major syn-dihydriodiol-epoxide-deoxyadenosine adduct formed increased with increasing concentrations of DMBA, the amounts of the major anti-dihydriodiol-epoxide adducts formed did not increase with DMBA concentration above 0.025 μg/ml. Thus the basal capacity of these tertiary cell cultures to generate anti-dihydriodiol-epoxide-DNA adducts was saturated at much lower DMBA concentrations than was their capacity to generate syn-dihydriodiol-epoxide-DNA adducts. As a result of this, the product distribution at 3 h was dependent on

<table>
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<th>Adducts</th>
<th>Total (μmol/mol DNA-P at 6 h)</th>
<th>anti-dGuo</th>
<th>syn-dAdo</th>
<th>anti-dAdo</th>
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</table>

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the DMBA concentration. At low DMBA concentrations, comparable amounts of each of the three major adducts were obtained while at the higher DMBA concentrations the major syn-dihydrodiol-epoxide-deoxyadenosine adduct was clearly the predominant product. At 6 and 24 h, the amounts of the anti-dihydrodiol-epoxide adducts increased in concert with DMBA concentration, presumably because the induction process had increased the capacity for anti-dihydrodiol-epoxide formation in most cases.

The extent of induction which occurred was reflected in the ratios of the amount of each adduct formed between 3 and 6 h relative to that formed between 0 and 3 h at various DMBA doses (Chart 8). For the syn-dihydrodiol-epoxide-deoxyadenosine adduct, this ratio was close to the value expected (1.0) in the absence of any induction at all DMBA concentrations. For the anti-dihydrodiol-epoxide-deoxyguanosine and -deoxyadenosine adducts, this ratio indicated that little induction of anti-dihydrodiol-epoxide-deoxyguanosine and -deoxyadenosine adducts, respectively.

DISCUSSION

In the studies described herein, several interrelated observations were made. In the tertiary cultures of fetal mouse cells in the absence of selenium, it was noted that the distribution of the major DMBA-deoxyribonucleoside adducts formed was dependent both on the time of exposure of the cultures to DMBA (see entries for controls in Tables 1 and 2 and Charts 4, 5, and 7) and on the concentration of DMBA to which the cells were exposed (Charts 7 and 8). For 6-h exposures, the three major adducts (anti-dihydrodiol-epoxide-deoxyguanosine and -deoxyadenosine and syn-dihydrodiol-epoxide-deoxyadenosine adducts) were present in roughly comparable amounts irrespective of the concentration of DMBA to which the cells had been exposed. After 3-h exposures of the cells to DMBA, however, comparable quantities of each of these three major adducts were obtained only with very low concentrations of DMBA (Chart 7).

At DMBA concentrations above 0.025 μg/ml, the syn-dihydrodiol-epoxide-deoxyadenosine adduct clearly was present in much greater quantities than at lower concentrations (Chart 7). The extent of induction of the syn-dihydrodiol-epoxide-deoxyadenosine adduct at 0.075 and 1.0 μg/ml was noted at the 0.01 μg/ml concentration of DMBA where selenium had no inhibitory effects (Chart 6). Induction then increased with increasing DMBA concentration until a maximum of a 6.5-fold increase in product formation was noted at the 0.075 and 1.0 μg/ml concentrations of DMBA.

In order to distinguish between the possibilities that selenite inhibited induced enzyme activity (36) or that selenite inhibited the induction process itself, the experiment summarized in Table 4 was undertaken. This study again confirmed the inhibitory effect of selenite administered 1 h before DMBA. The study also showed that when selenite is added 3 or 6 h after the DMBA, its inhibitory effects are substantially eliminated indicating that selenite is not an inhibitor of the induced enzyme system but that it inhibits the induction process itself. The inhibitory effect of selenite added 3 h after DMBA on binding at 12 h indicated that some induction occurs between 3 and 6 h.
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larger quantity than either anti-dihydrodiol-epoxide adduct (Charts 5 and 7).

These variations in adduct formation in the absence of selenium indicate that (a) the syn- and anti-dihydrodiol-epoxides are generated through two different enzyme systems; (b) the basal activity of these cells for the generation of syn-dihydrodiol-epoxide can handle much larger quantities of DMBA than can the activity for the generation of anti-dihydrodiol-epoxide which is saturated at quite low concentrations of DMBA (Chart 7); (c) DMBA or one of its metabolites triggers the induction of activity for the generation of the anti-dihydrodiol-epoxide but not the syn-diastereomer (Charts 4, 5, and 7); (d) concentrations of DMBA above 0.025 μg/ml are required for substantial induction (Chart 8); (e) the effects of this induction on DMBA binding to DNA are not apparent within the first 3 h of exposure to DMBA (Chart 5); and (f) this induction process is inhibited by actinomycin D, a well-known inhibitor of RNA synthesis (Table 3).

The effects of selenium on DMBA binding to DNA in these cells were of two types. At the highest dose of selenium (2 μg/ml) from sodium selenite or with most concentrations of selenodiglutathione a nonselective shut down of DMBA-DNA binding occurred. However, with more modest doses of sodium selenite, selective inhibition of the DMBA-DNA binding process occurred. This inhibition initially appeared to vary in a somewhat complex fashion but as the factors affecting the induction of anti-dihydrodiol-epoxide-forming activity were explored, it became clear that selenite was principally reducing that binding of DMBA to DNA which occurred through the induced anti-dihydrodiol-epoxide-forming activity. Thus under conditions where the induced activity did not contribute to DNA binding, i.e., within the first 3 h of exposure to DMBA or after 6 h of exposure to a very low concentration of DMBA, modest doses of selenium as sodium selenite had no effect on DMBA-DNA binding (Table 2; Charts 2, 4, and 6). Moreover where inhibition of DMBA-DNA binding was demonstrated, it was found that the modest doses of selenite selectively inhibited the formation of anti-dihydrodiol-epoxide-DNA adducts and had no effect on the syn-dihydrodiol-epoxide-DNA adducts. Since no induction of activity for the formation of syn-dihydrodiol-epoxide adducts was demonstrated, this again indicated that the effect of selenite was associated with the induced activity for forming the anti-dihydrodiol-epoxide.

The decreased levels of anti-dihydrodiol-epoxide-DNA adducts found in DNA from selenium-exposed cultures might be attributed to an increased excision repair of such adducts (21, 37) or to an increased detoxification of the anti-dihydrodiol-epoxide or perhaps to some general toxic effect. These kinds of mechanism, however, should have resulted in decreased yields of anti-dihydrodiol-epoxide adducts in the selenium-exposed cultures treated with DMBA (0.01 μg/ml) for 6 h (Chart 6) and this clearly did not occur. Thus the mechanism through which binding is inhibited by selenite presumably involves an inhibition of the induced activity (probably a cytochrome P-450 system) or inhibition of the induction itself. The effects of selenite observed here were mimicked by a known inhibitor of enzyme induction, actinomycin D (Table 3) and others have reported inhibitory effects of selenium on RNA synthesis (22, 38). The similarity between the effects of selenite and of actinomycin D is quite extensive, in fact, because Gelboin et al. (39) have shown that actinomycin D at a dose comparable to that used in these experiments is inhibitory of benz(a)anthracene-mediated induction of aryl hydrocarbon hydroxylase in cell cultures. It is also of interest that actinomycin D has been shown to inhibit DMBA-initiated tumorigenesis in mouse skin (40) as well as DMBA binding to DNA in mouse skin (unpublished results cited in Ref. 41). Perhaps the basis for this reduction of DNA binding in mouse skin is similar to that described in the cell cultures here because Bigger et al. (25) have shown that the adducts formed in mouse skin are the same as those formed in these fetal mouse cell cultures.

The alternate interpretation of a selective inhibition of an induced enzyme is not without precedent. Diamond and Gelboin (36) found that α-naphthoflavone selectively inhibited only induced aryl hydrocarbon hydroxylase activity in rat liver microsomes or hamster cell homogenates. This agent also dramatically inhibited the overall binding of DMBA to DNA in mouse skin, but it inhibited the formation of both anti- and syn-dihydrodiol-epoxide-DNA adducts to equal extents (34). It is clear therefore that selenite does not mimic the effects of α-naphthoflavone although this of itself does not exclude a selective inhibition of an induced cytochrome P-450 activity as a mechanistic basis for the effects of selenite described herein. The experiment summarized in Table 4 excludes this interpretation, however, since it shows that once DMBA has been allowed to induce the enzyme system, selenite is no longer inhibitory. The effects of selenite are attributable therefore to an inhibition of the induction process itself.

There are other reports in the literature indicating that selenium supplementation may modify the yield of various carcinogenic-reactive metabolites. For example, selenium can reduce the mutagenicity of a number of compounds requiring metabolic activation (42). Marshall et al. (19) have suggested that selenium may inhibit the carcinogenic activity of N-2-fluorenylacetamide by decreasing the yield of the N-hydroxylated metabolite and increasing the production of noncarcinogenic ring-hydroxylated metabolites and similar shifts in metabolism of DMBA have also been reported (43). Recently Smolarek and Baird (44) have shown that benzox(e)pyrene inhibits the binding of benzo(a)pyrene to DNA in hamster embryo cells and that adducts from the anti-dihydrodiol-epoxide are preferentially reduced. This study did not investigate the kinetic aspects of the inhibition, but the effects could possibly involve induction processes, as suggested herein. Indeed Sebti et al. (45) have found a somewhat increased contribution to DNA binding from the anti-dihydrodiol-epoxide of benzo(a)pyrene in various rodent cells between 5 and 48 h in culture which led them to consider the possibility of enzyme induction. In contrast to our findings, Ip and Daniel (20) have reported that dietary selenite does not affect DMBA binding to DNA in rat liver and mammary gland in vivo. These authors did not resolve individual syn- and anti-dihydrodiol epoxide adducts, however, so their findings do not totally exclude some modest selective inhibition of the nature described in this manuscript.

With respect to the broad problem of the mechanism through which selenium acts as a chemopreventive agent in chemical carcinogenesis, it should be remembered that our findings were obtained in an in vitro cell system, not in a target organ for DMBA carcinogenesis. Nevertheless our findings indicate that selenium could exert inhibitory effects on the metabolic activation of DMBA in vivo if the conditions of carcinogen administration were such that induction of a DMBA-activating enzyme system occurred. It is not probable, however, that this could account for
all of the chemopreventive action of selenium since it is inhibitory of DMBA-induced mammary carcinogenesis when administered long after DMBA as well as when administered at the same time as DMBA (15). It is possible, however, that selenium could be exerting its chemopreventive action through effects on gene expression. In this manuscript, we have documented the possible effect of selenium on the expression of one particular gene(s) (i.e., that responsible for formation of the anti-dihydriodiol-epoxide). It is possible that selenium could affect the expression of other genes, possibly of genes required for maintenance or establishment of the tumor phenotype.

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