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DNA Damage in L5178YS Cells following Exposure to Benzene Metabolites

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SUMMARY

Because DNA modification may be a prerequisite for chemical carcinogenesis, the DNA-damaging potential of benzene and its metabolites was examined in order to identify the proximate DNA-damaging agent associated with benzene exposure. A DNA synthesis inhibition assay previously identified *p*-benzoquinone as the most potent overall cellular toxin and inhibitor of DNA synthesis, but failed to discriminate among the hydroxylated metabolites. Therefore, the ability of benzene and its metabolites to induce DNA strand breaks in the mouse lymphoma cell line, L5178YS, was examined in order to provide a more accurate indication of the DNA damage associated with benzene and its metabolites. Cells were exposed to benzene, hydroquinone, catechol, phenol, 1,2,4-benzenetriol, or *p*-benzoquinone over a 1000-fold concentration range (1.0 μM–1.0 mM). Concentrations of benzene, phenol, or catechol as high as 1.0 mM did not increase the percentage of single-stranded DNA observed. Concentrations of hydroquinone as high as 0.1 mM were also ineffective. In contrast, both *p*-benzoquinone and 1,2,4-benzenetriol produced DNA breaks in a dose-related fashion. Of the two, benzoquinone proved to be more potent with an ED_{50} of ≈2.5 μM compared with 55.0 μM for benzenetriol. The DNA damage induced by 6.0 μM benzoquinone was maximal within 3 min of exposure and yielded approximately 70% single-stranded DNA after alkaline denaturation. By contrast, the single-stranded DNA observed after benzenetriol exposure required 60 min of exposure to achieve the same extent of damage as that found with benzoquinone. These results suggest that the benzene metabolites, benzenetriol and benzoquinone, may cause DNA damage and that the mechanisms responsible for the damage associated with these two compounds may be different.

Repeated exposure of humans to benzene is characterized by myelotoxicity which may be manifested in several ways including leukopenia, aplastic anemia, and, in the most serious cases, leukemia (1). The mechanism by which benzene induces leukemia is not understood. The observation that benzene caused chromosomal abnormalities in the bone marrow of occupationally exposed workers and experimentally exposed animals suggests that an interaction of benzene with nucleic acids may be involved (2, 3). Identification of radiolabel derived from a myelotoxic dose of ^{14}C-benzene covalently bound to nucleic acids of hematopoietic cells (4) provided additional evidence implicating nucleic acids as the critical targets associated with benzene-induced leukemia.

Evidence suggests that benzene bioactivation is a prerequisite for the myelotoxic effects. *In vitro* studies with human lymphocytes showed that, although benzene itself did not increase sister chromatid exchange or delay cell turnover, exposure to its metabolites, including catechol, hydroquinone, and phenol, resulted in significant clastogenicity (5). Moreover, Rushmore *et al.* (6) have provided evidence suggesting that the metabolites of benzene, including *p*-benzoquinone, phenol, hydroquinone, and 1,2,4-benzenetriol, form adducts with guanine following incubation of ^{14}C-benzene with bone marrow DNA. These observations suggest that the myelotoxic properties of benzene may be attributed to an interaction between benzene metabolites and DNA.

The metabolism of benzene has been investigated thoroughly and shown to be extensive. The microsomal cytochrome P-450-dependent monooxygenase systems mediate the oxidation of benzene to its primary metabolite, phenol, and to additional hydroxylation products including catechol, hydroquinone, and 1,2,4-benzenetriol (7, 8). The hydroxylated metabolites may be oxidized further by molecular oxygen to their corresponding quinones and semiquinone radicals generating reactive oxygen species in the process (9).

As a prerequisite to understanding the mechanism associated with benzene-induced leukemia, identification of the proximate toxin(s) resulting from benzene exposure is required. The DNA-damaging potential of benzene and its metabolites was screened in the mouse lymphoma cell line L5178YS (LY-S) (10) by the DNA synthesis inhibition assay designed by Painter (11). High concentrations of benzene had no inhibitory effect on DNA synthesis; however, the metabolites of benzene, including *p*-benzoquinone, hydroquinone, catechol, 1,2,4-benzenetriol, and phenol, all decreased ^{3}H-thymidine incorporation. Although this assay identified *p*-benzoquinone as the most potent ben-

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zene metabolite in inhibiting DNA synthesis, it failed to discriminate quantitatively among the hydroxylated metabolites. Therefore, as a more direct evaluation of the DNA damage imposed by benzene and its metabolites, we determined their ability to induce DNA strand breaks or alkaline-labile sites in LY-S cells. This investigation proved to be a more discriminating assessment of the DNA-damaging potential of benzene metabolites and distinguished benzenetriol and benzoquinone as DNA-damaging agents. Furthermore, data obtained from time course experiments suggest that benzenetriol- and benzoquinone-induced DNA damage may proceed via different mechanisms.

Materials and Methods

Chemicals and special equipment. Reagents required for cell culture were purchased from the following companies: Fisher's media, Gibco Laboratories, Grand Island, NY; streptomycin sulfate, Calbiochem-Behring, San Diego, CA; penicillin and L-glutamine, Sigma Chemical Co., St. Louis, MO; and horse serum, Hyclone, Logan, UT.

The benzene metabolites, phenol, *p*-benzoquinone, catechol, hydroquinone, and 1,2,4-benzenetriol, were purchased from Aldrich Chemical Co., Milwaukee, WI. They were the highest grade available and were used without further purification. Previous experiments comparing sublimed *p*-benzoquinone with that purchased from Aldrich Chemical Co. failed to detect a difference in their DNA-damaging potency (10).

Hydroxylapatite, fast flow, was obtained from Calbiochem-Behring. Apparatus required for hydroxylapatite chromatography including water-jacketed column rack and polystyrene columns (5.5 × 0.75 cm), were purchased from Isolab, Akron, OH. [*methyl*-^{3}H]Thymidine (specific activity 2 Ci/mmol) and Formula 963, an aqueous counting cocktail, were obtained from New England Nuclear, Boston, MA. [2-^{14}C] Thymidine (specific activity 56 mCi/mmol) and L-[4,5-^{3}H]leucine (specific activity 50 Ci/mmol) were obtained from ICN Pharmaceuticals, Inc., Irving, CA.

Cell culture. The mouse lymphoma cell line, LY-S, was used to assess the DNA-damaging potency and efficacy of benzene and its metabolites. This cell line was originally derived from a thymic tumor induced in the DBA/2 mouse by 3-methylcholanthrene (12). All experiments were performed using the X-ray sensitive strain, LY-S, isolated from the original X-ray-resistant strain in 1961 by Alexander (13). LY-S cells were kindly provided by Dr. H. Evans, Department of Radiation Biology.

LY-S cells were propagated as suspension cultures in growth media consisting of Fischer's medium supplemented with 10% horse serum, 2.4 mM glutamine, 100 units/ml of penicillin, and 183 μM streptomycin. Cultures of approximately 15 ml were carried in plastic flasks at 37° in a humidified 5% CO_2 atmosphere. To ensure optimal growth conditions, the cell density was maintained between 5 × 10^3 and 7 × 10^5 cells/ml.

Prelabeling of cells. LY-S cells were labeled with ^{3}H-thymidine (64.0 nCi/ml) or ^{14}C-thymidine (0.007 μCi/ml) for 18 hr prior to chemical exposure. Cold thymidine (0.16 μg/ml) was also added when labeling with ^{3}H-thymidine. Following the labeling period, cells were pelleted, washed, and incubated in nonradioactive media for at least 2 hr to prevent detection of unwinding from replication forks.

Toxicity. It is possible that the overall toxicity associated with the chemical may result in cell death. As such, DNA damage, when observed at chemical concentrations which also result in cell death, may be nonspecific and is unlikely to reflect those processes associated with heritable alterations in gene expression, i.e., mutagenesis or carcinogenesis. To circumvent any effects due to overall cytotoxicity associated with benzene and its metabolites, only doses of chemical which had no significant detrimental effects on cell viability were used to assess the DNA-damaging potential of benzene and its metabolites. Therefore, effects were identified as "DNA specific" when the percentage of single-stranded DNA was increased; but, a) membrane integrity remained ≧ 90% as assessed by trypan blue dye exclusion, and b) ^{3}H-leucine incorporation into acid-precipitable protein remained ≧ 90% of control. Procedures for evaluating trypan blue dye exclusion and protein synthesis were as described previously (10).

Chemical exposure. Immediately before the chemical exposure, cells were resuspended in Hanks' balanced salt solution at 2 × 10^5 cells/ml. Cells were then exposed to benzene or one of its metabolites. Exposures were carried out at 37° in a humidifed 5% CO_2 atmosphere unless otherwise stated. To terminate the chemical exposure, cells were pelleted, washed, and resuspended in ice-cold Hanks' balanced salt solution at 4 × 10^5 cells/ml. Approximately 6 × 10^4 cells were then transferred to conical tubes for alkaline treatment.

Measurement of DNA damage. In order to determine the potency and efficacy of benzene and its metabolites in causing alkali-labile sites and DNA strand breaks, we employed a modification of Rydberg's (14) alkaline denaturation method published by Sakai and Okada (15). Following exposure to the desired chemical, this procedure requires: 1) alkaline treatment to induce strand separation at breaks and alkali-labile sites, 2) neutralization to terminate strand separation, 3) sonication to prevent renaturation of the DNA strands, and 4) hydroxylapatite chromatography to separate the single- and double-stranded DNA. All procedures were performed as reported previously (14, 15). The percentage of single-stranded DNA is proportional to the number of breaks in the DNA phosphodiester chain (15). Henceforth, DNA damage will be discussed in terms of the percentage of single-stranded DNA observed following alkaline denaturation of chemically treated cells.

Quantitation of DNA damage. The single- and double-stranded DNA in a given sample was eluted from hydroxylapatite columns using two 2.0-ml portions of 0.125 M potassium phosphate buffer (KPi), pH 6.8, followed by two 2.0-ml aliquots of 0.25 M KPi buffer, pH 6.8. To each 2.0-ml fraction eluted from the column, 15 ml of Formula 963 liquid scintillation fluid were added, and radioactivity was quantitated on a Beckman model LS3801 liquid scintillation counter. Counting efficiencies were routinely 40 and 75% for ^{3}H and ^{14}C, respectively. The percentage of single-stranded DNA (% S.S.DNA) in a given sample was calculated according to Eq. 1:

$$\frac{\text{cpm } 0.125 \text{ M KPi}}{\text{cpm } 0.25 \text{ M KPi} + \text{cpm } 0.125 \text{ M KPi}} \times 100 = \%\ \text{S.S.DNA} \quad (1)$$

The percentage of single-stranded DNA attributed specifically to chemical exposure was calculated according to Eq. 2. Both ^{3}H and ^{14}C labeling were used to distinguish chemical effects from any effects associated with the isotope (15).

$$\%\ \text{S.S.DNA} = 100 \times \frac{(\%\ \text{S.S.DNA}\ ^{14}\text{C treated} - \%\ \text{S.S.DNA}\ ^{14}\text{C control}) + (\%\ \text{S.S.DNA}\ ^{3}\text{H-treated} - \%\ \text{S.S.DNA}\ ^{3}\text{H control})}{(\%\ \text{S.S.DNA}\ ^{14}\text{C denatured} - \%\ \text{S.S.DNA}\ ^{14}\text{C control}) + (\%\ \text{S.S.DNA}\ ^{3}\text{H denatured} - \%\ \text{S.S.DNA}\ ^{3}\text{H control})} \quad (2)$$

Time dependence of single-strand break formation. The standard conditions established for the 30-min exposure described above require that the exposures be conducted in a 5% CO_2, 37° incubator. However, in order to determine the dependence of exposure time on the production of DNA damage, the protocol was modified slightly. In these experiments, cells were preequilibrated for 15 min in HBSS at 37° in a 5% CO_2 incubator, drug added, and an aliquot removed at the designated time. With the shorter exposure time, i.e., 0, 1, and 2 min, the cells remained at room temperature since it was not technically feasible to obtain these time points if cells were returned to the incubator.

Calculations. The dose-response curves were analyzed according to the procedure of DeLean *et al.* (16). The ED_{50} values were estimated using a nonlinear regression program, NONLIN (17).

Results

Toxicity. The highest doses of benzene and its metabolites which routinely yielded ≧ 90% viability according to trypan blue dye exclusion and protein synthesis are reported in Fig. 1. Concentrations of benzene, phenol, and catechol as high as 1.0 mM had no effect on ^{3}H-leucine incorporation. In contrast, inhibitory effects on protein synthesis were observed after a 30-min incubation with 0.1 mM benzenetriol or 0.06 mM hydroquinone. Benzoquinone was the most potent inhibitor of protein synthesis.

Determination of cell viability based upon trypan blue dye exclusion yielded results similar to those obtained for protein synthesis. Treatment with 1.0 mM benzene, phenol, or catechol had no effect on membrane integrity. Only the most potent metabolites in inhibiting protein synthesis, i.e., benzoquinone, hydroquinone, and benzenetriol, had detrimental effects on membrane integrity at lower concentrations.

Production of single-stranded DNA. Table 1 shows the percentage of single-stranded DNA obtained following a 30-min exposure of LY-S cells to the highest nontoxic dose of benzene or its metabolites. Doses of benzene, phenol, or catechol as high as 1.0 mM or 0.1 mM hydroquinone did not significantly increase the percentage of single-stranded DNA observed. In contrast, both *p*-benzoquinone and 1,2,4-benzenetriol were capable of inducing DNA strand breaks. Between 65 and 73% single-stranded DNA was observed following a 30-min treatment with 4.0–6.0 μM benzoquinone. Similarly, exposure to 50–60 μM benzenetriol yielded between 43 and 59% single-stranded DNA.

Results obtained for benzenetriol- and benzoquinone-induced DNA damage are presented for a narrow range of low concentrations regularly associated with ≧90% viability. Due to the rigorous standards established for assessing viability and the variability encountered, especially when working within narrow concentration ranges, it was difficult to accurately define an absolute value for the "highest nontoxic dose" associated with benzoquinone and benzenetriol. Similar problems did not arise with benzene and the other metabolites since high concentrations did not produce DNA damage. Hence, it was not necessary to investigate them at lower and more narrow concentration ranges where subtle changes in toxicity are often difficult to discern.

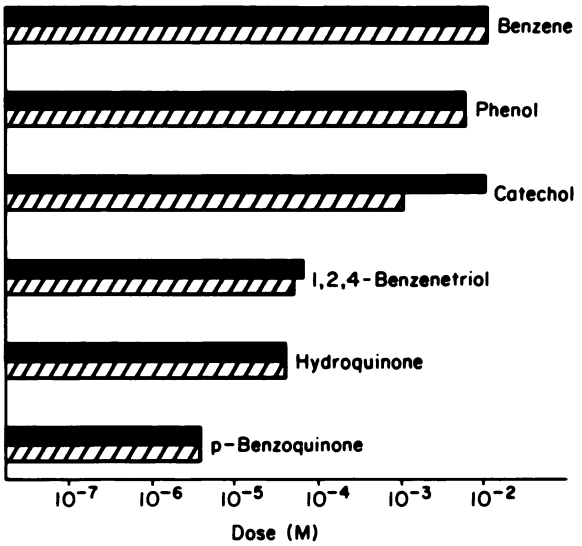

Fig. 1. Highest nontoxic dose of benzene and metabolites routinely obtained following their 30-min exposure to LY-S cells as described in Materials and Methods. ■, highest nontoxic dose according to trypan blue dye exclusion; ▨, highest nontoxic dose according to protein synthesis. Results are based upon data obtained from at least three individual experiments.

TABLE 1

Percentage of single-stranded DNA in LY-S cells following exposure to the highest nontoxic dose of benzene and its metabolites

Chemical	*n*[a]	Dose	Percentage of single stranded DNA[b]
		M	
Benzene[c]	3	1.0×10^{-3}[d]	0
Phenol	2	1.0×10^{-3}[d]	1–3
Catechol	4	1.0×10^{-3}	0–7
Hydroquinone	4	1.0×10^{-4}	0–7
1,2,4-Benzenetriol	14	$5.0–6.0 \times 10^{-5}$	43–59
Benzoquinone	32	$4.0–6.0 \times 10^{-6}$	65–73

[a] *n*, the number of individual experiments performed for the dose or range of doses stated. An individual experiment includes results obtained with both ^{14}C- and ^{3}H-labeled cells.

[b] LY-S cells were treated for 30 min with the specified dose of benzene or metabolites. Following the exposure cells were treated with alkali to induce strand separation after which single- or double-stranded DNA was separated usng hydroxylapatite chromatography. The percentage of single-stranded DNA was calculated as described in Materials and Methods.

[c] Benzene was initially administered to yield a final concentration of 1.0 mM in the reaction media. Because of the volatility of benzene, the concentration may have decreased up to 50% during the exposure.

[d] Although higher doses were shown to be nontoxic, they were not investigated since it is unlikely that higher concentrations would be achieved under physiologic conditions.

The percentage of single-stranded DNA observed following a 30-min treatment with either benzoquinone or benzenetriol was dose related as demonstrated in Fig. 2. Benzoquinone was approximately 20-fold more potent than benzenetriol in producing DNA strand breaks with ED_{50} values of 2.5 μM and 55.0 μM, respectively.

Relationship between the time of exposure and percentage of single-stranded DNA. Under the conditions established for our routine determinations, benzoquinone appeared to be approximately 20 times more potent and 1.23 times more effective than benzenetriol in producing alkali-labile sites and single-stranded DNA. In order to determine whether benzenetriol was intrinsically less active than benzoquinone or whether this merely reflected differences in the rates of interactions by the two metabolites, the effect of time of exposure on production of DNA damage was examined.

As shown in Fig. 3, the percentage of single-stranded DNA observed following incubation with either benzoquinone or benzentriol appeared to be dependent on time of exposure. The onset of DNA damage following benzoquinone treatment was extremely rapid. After a 1.0-min exposure to 6.0 μM benzoquinone, 55% of the DNA was single stranded. The effect was maximal by 3.0 min, yielding approximately 60% single-stranded DNA. Continuing the incubation for an additional 60 min did not substantially increase the percentage of single-stranded DNA observed. In contrast, the production of single-stranded DNA with benzenetriol required longer exposure times. Maximal DNA damage was not evident until 60 min after exposure to 60 μM benzenetriol when the percentage of single-stranded DNA leveled off at 80%.

The prolonged exposure time required to produce DNA dam-

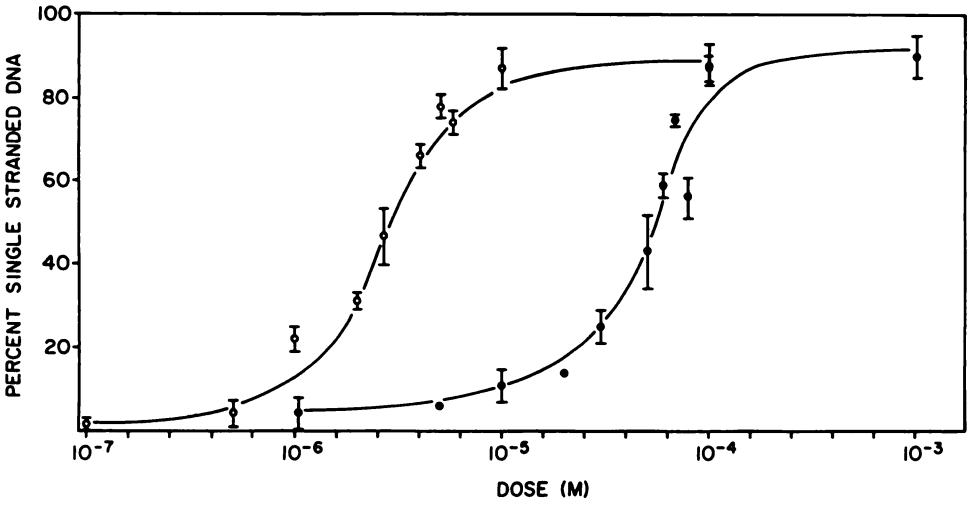

Fig. 2. Percentage of single-stranded DNA observed following alkaline denaturations of LY-S cells treated for 30 min with various doses of benzenetriol (●) or benzoquinone (○). Chemical treatment, alkaline denaturation, and hydroxylapatite chromatography were performed as described in Materials and Methods. All *points* represent the mean ± standard errors of at least three independent experiments except for 5.0 and 20.0 μM benzenetriol which were investigated in only one experiment.

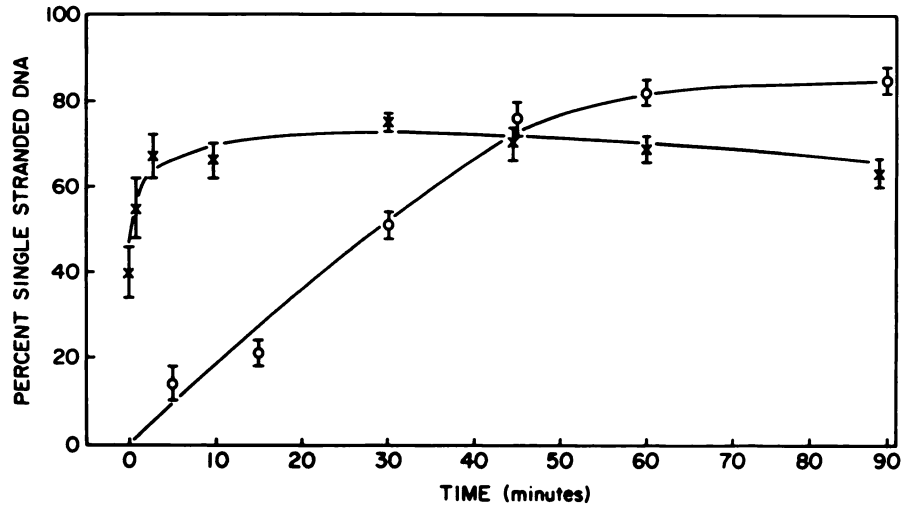

Fig. 3. Percentage of single-stranded DNA observed following alkaline denaturation of LY-S cells exposed to 60.0 μM benzenetriol (○) or 6.0 μM benzoquinone (*x*) for the designated time. Procedures were described in Materials and Methods. Results demonstrate the mean ± standard errors of at least three separate experiments.

age with benzenetriol prompted us to investigate a lower dose of benzenetriol for longer periods of time. Since the ED_{50} determined for benzenetriol was based upon a 30-min exposure, it is possible that benzenetriol would appear more potent if examined over a longer period of time. Fig. 4 demonstrates the results obtained following prolonged treatment of LY-S cells to 30.0 μM benzenetriol. Whereas this dose showed only minimal damaging effects on DNA following a 30-min exposure, a maximal level of 80% single-stranded DNA was observed following a 90-min exposure.

Effect of temperature on benzoquinone-induced DNA damage. The high percentage of single-stranded DNA observed at the zero time point for benzoquinone made it difficult to determine whether benzoquinone did exhibit a true time-dependent effect or whether the rapid onset of DNA damage was an artifact of the system. Therefore, the time course experiments were repeated at 4°. As shown in Fig. 5, decreasing the temperature to 4° delayed the onset of DNA damage substantially; the percentage of single-stranded DNA observed at the zero time point was reduced to 4% compared to 40% obtained at 37°. The decrease in temperature did not change the efficacy of benzoquinone since the maximal percentage of single-stranded DNA obtained was approximately 60% at both 4° and 37°. Similarly, maximal DNA damage was observed

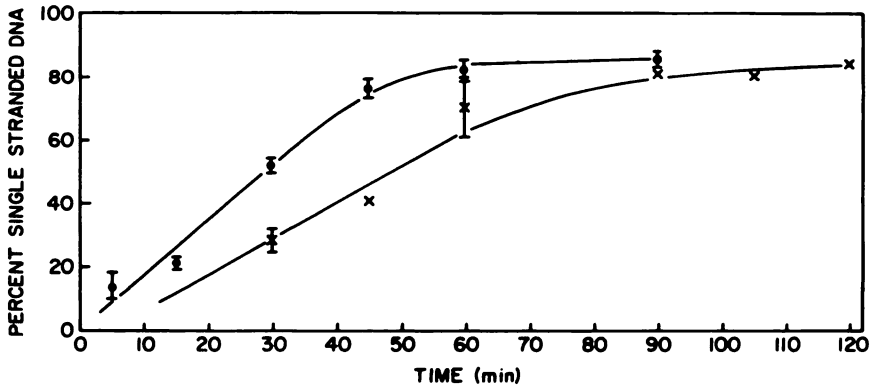

Fig. 4. Percentage of single-stranded DNA obtained following alkaline treatment of LY-S cells exposed to 60.0 (●) or 30.0 μM benzenetriol (*x*) for the designated time. Procedures were as described in Materials and Methods. Results demonstrate the mean ± standard errors of at least three separate experiments except where *error bars* are excluded for 30.0 μM benzenetriol which reflect the data obtained from one experiment.

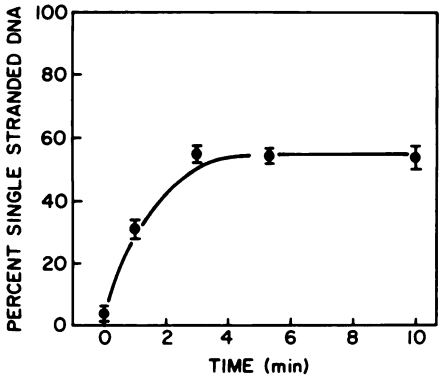

Fig. 5. Percentage of single-stranded DNA observed following alkaline denaturation of LY-S cells exposed to 6.0 μM benzoquinone on ice for the designated time. Exposure, denaturation, and chromatography were performed as described in Materials and Methods. Results reflect the mean ± standard errors of at least three separate trials.

following a 3-min exposure to benzoquinone at both temperatures.

The time-dependent production of DNA damage following benzoquinone and benzenetriol treatment prompted us to examine hydroquinone and catechol for additional periods of time. Treatment of LY-S cells with 0.1 mM hydroquinone or 1.0 mM catechol, the highest nontoxic dose, for 1 hr or more did not result in increased production of single-stranded DNA.

Discussion

During the past decade, an abundance of evidence has accumulated suggesting that reactions with cellular DNA may be prerequisite to the mutagenicity and/or carcinogenicity of many chemicals (18, 19). With this in mind, we studied the DNA damage associated with exposure of LY-S cells to benzene and its metabolites.

Our experimental system, the LY-S cell, was chosen both because of its lymphoid origin and because of its inability to hydroxylate benzene. Such a system permitted the toxic effects of benzene to be distinguished from those of its metabolites. Most previous investigators have used whole animals or microsomal systems which are competent in benzene hydroxylation and make it difficult to distinguish the effects of benzene alone from those of one or more of its metabolites (5, 7, 20, 21).

Results of our investigation have identified *p*-benzoquinone and 1,2,4-benzenetriol as the metabolites of benzene capable of inducing single-stranded DNA in LY-S cells. *p*-Benzoquinone was distinguished as the most potent metabolite in inducing the single-stranded DNA which is consistent with our previous report demonstrating that *p*-benzoquinone was the most potent cellular toxin and inhibitor of DNA synthesis in LY-S cells. Benzoquinone and benzenetriol were also identified by Schwartz *et al.* (22) as the only benzene metabolites to inhibit rat liver mitochondrial DNA synthesis.

It is interesting to consider the ED_{50} values for benzoquinone-induced inhibition of DNA synthesis (10) and induction of single-stranded DNA, which were 5.0 μM and 2.5 μM, respectively. The ED_{50} values obtained for inhibition of DNA synthesis and induction of single-stranded DNA with benzenetriol were 180 μM and 55 μM, respectively. In both assays, the production of single-stranded DNA appeared to be a more sensitive indicator of DNA damage.

Results obtained from varying the time of exposure on the percentage of single-stranded observed DNA suggest that benzenetriol and benzoquinone might act through different mechanisms to induce the damage. The rapid onset of DNA damage following exposure to *p*-benzoquinone suggests that metabolic activation may not be required for generating the DNA-damaging species. Rather, the DNA damage may be a consequence of a direct interaction between benzoquinone and DNA. Because of its electrophilic nature (23), benzoquinone may react directly with nucleophilic centers in DNA via alkylation. The detection of adducts between benzoquinone and guanine upon incubation of bone marrow mitoplasts with ^{14}C-benzene provide further support for this hypothesis (6).

In contrast to the results obtained with benzoquinone, the DNA damage resulting from benzenetriol exposure demonstrated a significant time dependence. Although it is conceivable that the observed time dependence is reflective of slow uptake of benzenetriol by the cells, published partition coefficient values indicate that benzoquinone is less hydrophobic than the hydroxylated metabolites (24). Therefore, the latter is more likely to demonstrate slow uptake than benzenetriol. Since benzoquinone shows a maximum effect in only 3 min, it is unlikely that the time dependence of benzenetriol is reflective of slow cellular uptake.

The slow onset of benzenetriol-induced DNA damage could reflect its rate of oxidation to reactive quinones. Hydroxylated phenols can be oxidized to their corresponding quinones by sequential one-electron or direct two-electron oxidations. Such is the case for the oxidation of catechol by tyrosinase where the ESR spectrum of an *o*-benzosemiquinone is readily observable in the catechol/peroxidase/H_2O_2 reaction, but not in the tyrosinase-catalyzed reaction (25). As proposed for benzoquinone, the quinone of benzenetriol might form covalent adducts with nucleophilic sites in DNA.

Alternatively, the DNA damage observed following benzenetriol exposure may result from reactive oxygen species generated during its autooxidation. The oxidation of benzenetriol and related hydroxylated metabolites, and the generation of reactive intermediates, has been presented by Greenlee *et al.* (9). As shown in Fig. 6, benzenetriol can be oxidized by molecular oxygen to a semiquinone with the concomitant generation of superoxide radicals. The semiquinone can be enzymatically reduced by NADPH-cytochrome P-450 reductase back to benzenetriol or, alternatively, can be further oxidized to the quinone, again accomplished with superoxide formation. Cycling between the semiquinone and benzenetriol or its quinone would provide a mechanism for continued superoxide formation. Superoxide radicals may further react with hydrogen peroxide to yield hydroxyl radicals which are considered to be responsible for the majority of DNA damage induced *in vitro* by ionizing radiation (26, 27).

A redox-type mechanism has been implicated in DNA damage associated with various quinone-containing antineoplastic agents including adriamycin and streptonigrin (28, 29). Chemical reduction has been shown to activate adriamycin to a semiquinone-free radical and induce single-strand scission of PM2 DNA (28). Gutteridge (29) reported that the production of DNA strand cleavage by streptonigrin reduced *in situ* by NADPH was dependent on oxygen and completely inhibited by superoxide dismutase, suggesting the intermediacy of the superoxide radical in the degradation. Similar type mechanisms may be involved in the DNA damage induced by benzenetriol.

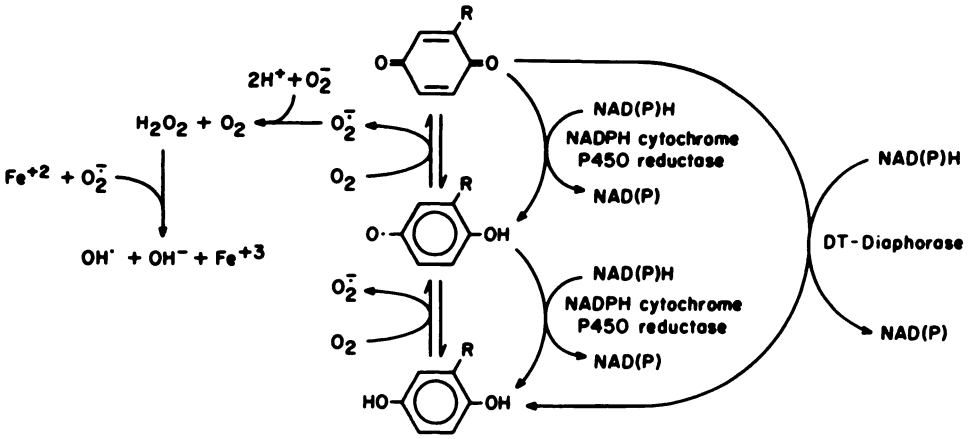

Fig. 6. Potential toxification and/or detoxification pathways for benzoquinone and benzenetriol via redox cycling between the quinone and its hydroxylated counterpart. Reaction scheme also demonstrates the concomitant production of reactive oxygen species. Benzoquinone, *R* = H; benzenetriol, *R* = OH.

The ability to detect DNA damage following exposure of LY-S cells to benzoquinone but not hydroquinone suggests that, within our experimental conditions, hydroquinone is not appreciably oxidized to benzoquinone. The striking difference in the DNA damage produced by benzenetriol and hydroquinone may reflect differences in the kinetics of hydroquinone and benzenetriol oxidation. Greenlee *et al.* (9) provided evidence to suggest that the autooxidation of benzenetriol and hydroquinone may proceed by different mechanisms. Whereas superoxide dismutase inhibited benzenetriol-induced oxidation of epinephrine, it enhanced hydroquinone-induced oxidation of epinephrine. The oxidation of benzenetriol and hydroquinone in LY-S cells may also proceed through different mechanisms or at different rates which may explain the inability to detect strand breaks following hydroquinone exposure.

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