

Benzene and Its Phenolic Metabolites Produce Oxidative DNA Damage in HL60 Cells *in Vitro* and in the Bone Marrow *in Vivo*¹

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ABSTRACT

Benzene, an important industrial chemical, is myelotoxic and leukemogenic in humans. It is metabolized by cytochrome P450 2E1 to various phenolic metabolites which accumulate in the bone marrow. Bone marrow contains high levels of myeloperoxidase which can catalyze the further metabolism of the phenolic metabolites to reactive free radical species. Redox cycling of these free radical species produces active oxygen. This active oxygen may damage cellular DNA (known as oxidative DNA damage) and induce genotoxic effects. Here we report the induction of oxidative DNA damage by benzene and its phenolic metabolites in HL60 cells *in vitro* and in the bone marrow of C57BL/6 × C3H F₁ mice *in vivo* utilizing 8-hydroxy-2'-deoxyguanosine as a marker. HL60 cells (a human leukemia cell line) contain high levels of myeloperoxidase and were used as an *in vitro* model system. Exposure of these cells to phenol, hydroquinone, and 1,2,4-benzenetriol resulted in an increased level of oxidative DNA damage. An increase in oxidative DNA damage was also observed in the mouse bone marrow *in vivo* 1 h after benzene administration. A dose of 200 mg/kg benzene produced a 5-fold increase in the 8-hydroxydeoxyguanosine level. Combinations of phenol, catechol, and hydroquinone also resulted in significant increases in steady state levels of oxidative DNA damage in the mouse bone marrow but were not effective when administered individually. Administration of 1,2,4-benzenetriol alone did, however, result in a significant increase in oxidative DNA damage. This represents the first direct demonstration of active oxygen production by benzene and its phenolic metabolites *in vivo*. The conversion of benzene to phenolic metabolites and the subsequent production of oxidative DNA damage may therefore play a role in the benzene-induced genotoxicity, myelotoxicity, and leukemia.

INTRODUCTION

Chronic exposure to benzene, an ubiquitous pollutant, induces myelotoxicity and leukemia in humans (reviewed in Ref. 1). However, the precise mechanisms by which benzene induces these effects are not known. Various studies have, however, shown that benzene metabolism is required to exert its myelotoxic effects (reviewed in Refs. 2 and 3). The majority of benzene metabolism occurs in liver, where cytochrome P-450 oxidizes benzene to PH,⁵ CAT, HQ, BT, and a ring-opened product called *trans,trans*-muconaldehyde, which is oxidized to *trans,trans*-muconic acid. These phenolic metabolites and muconic acid accumulate in the bone marrow (4, 5). MPO and other heme-protein peroxidases present in the bone marrow may further

convert the phenolic metabolites of benzene to reactive semiquinones and quinones (6-10). These semiquinones, quinones, and, perhaps, *trans,trans*-muconaldehyde are generally believed to be the ultimate toxic species derived from benzene.

Benzene and various combinations of its metabolites produce genetic damage, including numerical and structural chromosomal aberrations in blood and bone marrow of exposed animals and humans (11-16). One mechanism by which benzene induces these genotoxic effects may be by generating one or more active oxygen species such as superoxide anion radicals (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radicals (HO[•]), and singlet oxygen (¹O₂) in the bone marrow (6). Active oxygen could be generated by peroxidatic metabolism or autoxidation of phenolic metabolites in the bone marrow (6, 8, 17). Previous *in vitro* studies have shown that semiquinone radicals formed during the peroxidatic metabolism of HQ can reduce dioxygen to O₂⁻ (8) and have the potential to undergo cyclic reduction and oxidation reactions (redox cycling) to produce large amounts of active oxygen. However, the ability of benzene or its phenolic metabolites to induce the formation of active oxygen *in vivo* and cause damage to cellular DNA and proteins has not been studied. The primary objective of the present study was to test this hypothesis.

Recently, Kasai and Nishimura (18) and Floyd *et al.* (19) have shown that active oxygen including HO[•] and ¹O₂ can hydroxylate deoxyguanosine residues in DNA resulting in the formation of 8OHdGua. This 8OHdGua can be easily detected by HPLC with electrochemical detection, following enzymatic hydrolysis of DNA. This method has become a useful tool to measure the formation of active oxygen in complex biological systems (such as *in vivo* situations) where active oxygen cannot be directly detected (20-22). In addition, the detection of 8OHdGua in DNA provides a direct measure of genotoxic effects of active oxygen (20). Therefore, in this study, we have utilized 8OHdGua formation in DNA as a marker for the detection of active oxygen formation. Initial studies were performed *in vitro* in human myeloid leukemia (HL60) cells by exposing them to the phenolic metabolites of benzene, because these cells contain high levels of MPO (23). Further studies were performed in C57BL/6 × C3H F₁ (hereafter called B6C3F₁) mice exposed to benzene or its phenolic metabolites. The data show that benzene or its phenolic metabolites produce oxidative DNA damage *in vitro* in HL60 cells and *in vivo* in bone marrow (the target organ for benzene) of B6C3F₁ mice. This study provides the first direct evidence for the production of active oxygen *in vivo* by benzene.

MATERIALS AND METHODS

Reagents. Benzene (99.9% pure) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Phenol, hydroquinone, catechol, 1,2,4-benzenetriol, nuclease P₁ and alkaline phosphatase (*Escherichia coli*) were purchased from Sigma Chemical Co. (St. Louis, MO). High purity distilled phenol was purchased from International Biotechnologies, Inc. (New Haven, CT). All other chemicals and solvents were of the highest grade commercially available.

Standard Cell Culture Conditions. HL60 cells obtained from the American Type Culture Collection (Rockville, MD) were cultured in RPMI 1640 supplemented with fetal bovine serum albumin (10%) and gentamicin sulfate (50 µg/ml). Cells were grown in a humidified atmosphere in 5% CO₂ and 37°C. Cell viability was determined using trypan blue exclusion in which 200 cells/culture were analyzed. All initial viabilities were greater than 95%.

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⁵ The abbreviations used are: PH, phenol; BT, 1,2,4-benzenetriol; CAT, catechol; dGua, deoxyguanosine; HPLC, high pressure liquid chromatography; HQ, hydroquinone; 8OHdGua, 8-hydroxy-2'-deoxyguanosine; MPO, myeloperoxidase; PBS, phosphate-buffered saline; DMSO, dimethyl sulfoxide.

In Vitro Studies. HL60 cell cultures were preincubated for 0.5 h at 37°C before dosing with various metabolites of benzene. Cells ($0.5 \times 10^6/\text{ml}$) were incubated in PBS (20 ml; pH 7.4) with HQ, PH, CAT, or BT ($10\text{--}100\ \mu\text{M}$) up to 1 h. The cells were pelleted by centrifugation (2000 rpm/5 min) and washed twice with PBS prior to DNA extraction.

Assay for MPO Activity in HL60 Cells. Myeloperoxidase activity in HL60 cells was determined using 3,3'-tetramethylbenzidine as a substrate as described previously by Bozeman *et al.* (24). With this method, we found that HL60 cells contain $74 \pm 26\ \text{pmol}$ of MPO/ 10^6 cells.

In Vivo Studies. Male B6C3F₁ mice (25–30 g, about 6 weeks old) were purchased from Simonsen Laboratories (Gilroy, CA) and housed 1–2 weeks prior to use. Mice were fed standard Purina Rat Chow 5012 and water *ad libitum* and were maintained in a temperature and photoperiod (12 h/day)-controlled room. The animals were housed 1/cage.

Benzene Studies. Benzene dissolved in corn oil was used for all studies. Time course studies were performed using a single dose (880 mg/kg) of benzene administered i.p. to 4 groups of mice (3 mice/group). Each treatment group had corresponding simultaneous vehicle controls (3 mice/group). All animals were euthanized at 1, 3, 6, or 12 h after treatment by cervical dislocation. Dose-response studies were performed using 6 groups of mice (3 mice/group) given i.p. injections of single doses of 0, 50, 100, 200, 400, or 800 mg/kg of benzene. All animals were euthanized 1 h after treatment by cervical dislocation.

Phenolic Metabolite Studies. Phenolic metabolites of benzene were dissolved in PBS. Nine groups of mice (3 mice/group) were given i.p. injections of PH (75 mg/kg), HQ (75 mg/kg), CAT (75 mg/kg), BT (25, 50, or 75 mg/kg), or a combination of PH + HQ (75 + 75 mg/kg), HQ + CAT (75 + 75 mg/kg) and PH + CAT (75 + 75 mg/kg). An additional group of 3 mice received an equivalent amount of PBS alone. All the chemicals were preweighed and the solutions were prepared immediately before administration to avoid autoxidation. All animals were euthanized 1 h after treatment by cervical dislocation.

Isolation of Bone Marrow Cells. Bone marrow cells were isolated from mouse femurs (9) into 3 ml of ice-cold PBS (pH 7) containing 0.16% (w/v) EDTA. The cells were dispersed by gentle aspiration with a Pasteur pipet, centrifuged at $150 \times g$ for 15 min, and resuspended in the same buffer. Approximately $40\text{--}50 \times 10^6$ bone marrow cells (pooled from 2 femurs) were harvested from each mouse by this method.

Determination of 8OHdGua in DNA. DNA was isolated from mouse bone marrow and HL60 cells by the phenol extraction procedure of Gupta *et al.* (25). To avoid any additional oxidative damage to the DNA due to peroxide or quinone contaminants in phenol, high purity double distilled phenol was used for extractions. About 200–400 μg DNA were resuspended in 200 μl 20 mM sodium acetate (pH 4.8), and digested to nucleotides with 20 μg nuclease P₁ at 70°C for 15 min. To adjust the pH 20 μl of 1 M Tris-HCl (pH 7.4) were added to the nucleoside mixture, which then was treated with 1.3 units of *E. coli* alkaline phosphatase and incubated at 37°C for 60 min. These hydrolyzed DNA solutions were then filtered using an Ultrafree Millipore filtration system (10,000-dalton cutoff). The HPLC conditions used in the present study have been described previously (26). Briefly, the amount of 8OHdGua present in the DNA was analyzed by flow-through electrochemical detection using an ESA model 5100 Coulochem detector equipped with a 5011 or 5100 high sensitivity analytical cell with the oxidation potentials of electrodes 1 and 2 adjusted to 0.10 and 0.35 V, respectively. A Supelco LC-18 HPLC column (15 $\times$ 4.6 mm) was utilized for separation of 8OHdGua. The mobile phase consisted of 10% methanol and 50 mM KH₂PO₄ buffer, pH 5.5, run isocratically at a flow rate of 1 ml/min.

Synthesis of 8OHdGua Standard. Standard 8OHdGua was synthesized by Udenfriend's hydroxylating system as described by Kasai and Nishimura (27). Hydroxylation of dGua at the C-8 position was carried out by sequential addition of 25 μl of 0.1 M dGua, 14 μl of 1 M ascorbic acid, 6.5 μl of 1 M EDTA, and 13 μl of 0.13 M FeSO₄ to 0.942 ml of 0.1 M sodium phosphate buffer (pH 6.8) and incubating up to 15 min at 37°C in the dark with vigorous shaking. Following incubation, aliquots of the reaction mixture were analyzed by HPLC. Fractions containing 8OHdGua were collected from HPLC eluates, lyophilized, and stored at 4°C for use as standards.

Statistics. All results are expressed as mean $\pm$ SD of at least three experiments. *P* values for significance were determined using the two-tailed Student's *t* test.

RESULTS

Induction of Oxidative DNA Damage by Phenolic Metabolites *in Vitro* in HL60 Cells

Table 1 shows the effect of different benzene metabolites on the formation of 8OHdGua in the DNA of HL60 cells. HQ ($10\ \mu\text{M}$) induced a 2-fold increase in 8OHdGua levels after 30 min of incubation, while $100\ \mu\text{M}$ PH produced a 3.5-fold increase. The most effective inducer of 8OHdGua formation was BT, a minor metabolite of benzene, which produced a maximum of 5-fold increase in 8OHdGua after 30 min. No significant increase in 8OHdGua level was observed, even after 1 h incubation, with CAT ($100\ \mu\text{M}$).

Little or no cytotoxicity was observed from exposure to these compounds for at least 6 h (assayed by trypan blue exclusion test; data not shown), indicating that 8OHdGua formation in these cells does not occur after cell death. In summary, HQ, PH, and BT, but not CAT, induce rapid 8OHdGua formation in HL60 cells that returns to normal levels following further incubation presumably due to the rapid repair of DNA damage.

Oxidative DNA Damage *in Vivo* in Mouse Bone Marrow following Exposure to Benzene

Time Course. A rapid, 2-fold increase [$P < 0.001$] in 8OHdGua levels in mouse bone marrow DNA was observed 1 h after benzene administration [880 mg/kg (Fig. 1)]. This increase gradually declined towards background levels after 6 h ($P > 0.1$) and remained constant through 12 h. As shown in Fig. 1, the vehicle (corn oil) had no observable effect on the steady state levels of 8OHdGua throughout treatment. The maximum oxidative damage by benzene was observed after 1 h, and this time point was therefore chosen for additional studies. These results further show that maximum oxidative damage occurs well before cytotoxicity (none prior to 12 h) (28) and may contribute to but is not a consequence of toxicity.

Dose Response. The effect of different benzene doses on 8OHdGua levels in mouse bone marrow is shown in Fig. 2. A significant increase ($P < 0.05$) in oxidative DNA damage was observed even with the lowest dose, 50 mg/kg (Fig. 2), reaching a maximum of 5-fold increase ($P < 0.001$) in 8OHdGua levels at 200 mg/kg. Higher doses of benzene resulted in smaller increases in the 8OHdGua level, about 2.5-fold ($P < 0.001$) above control levels at 400 mg/kg and only 2-fold ($P < 0.001$) at 800 mg/kg.

Oxidative DNA Damage *in Vivo* in Mouse Bone Marrow following Exposure to Phenolic Metabolites of Benzene

When administered alone, PH (75 mg/kg), CAT (75 mg/kg), or HQ (75 mg/kg) had no significant effect on the steady state level of 8OHdGua (Fig. 3), which ranged from 0.045 to 0.053 pmol. However, when mice were dosed with a combination of HQ (75 mg/kg) + PH (75 mg/kg) or with a combination of HQ (75 mg/kg) + CAT (75 mg/kg), a 2-fold increase ($P < 0.01$) in the 8OHdGua level over the control value was observed (Fig. 3). The combination of PH (75

Table 1 Oxidative DNA damage in HL60 cells by benzene metabolites, hydroquinone, phenol, catechol, and 1,2,4-benzenetriol

Incubations were performed for 30 min as described in "Materials and Methods." Results are expressed as mean $\pm$ SD of 3 experiments.

Phenolic substrate	pmol 8OHdGua/ μg DNA
None	0.080 ± 0.025
HQ ($10\ \mu\text{M}$)	0.160 ± 0.030^a
Phenol ($100\ \mu\text{M}$)	0.270 ± 0.032^a
CAT ($100\ \mu\text{M}$)	0.100 ± 0.012^b
1,2,4-Benzenetriol ($10\ \mu\text{M}$)	0.310 ± 0.018^a

^a $P < 0.05$; significantly different from controls (none).

^b $P > 0.05$; not significantly different from controls (none).

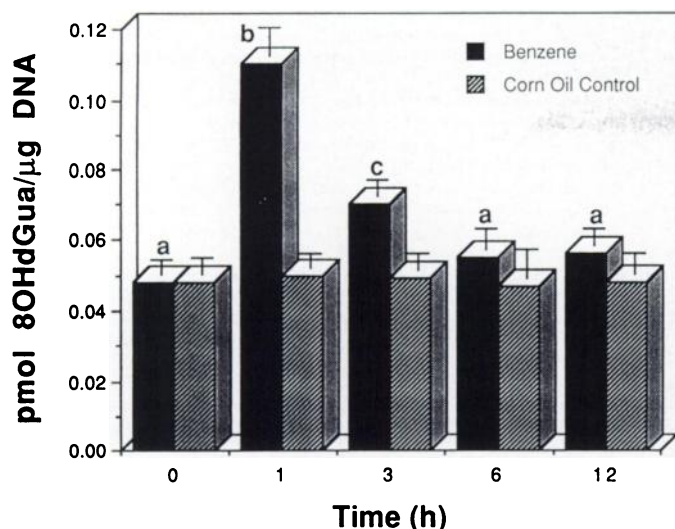


Fig. 1. Time course of benzene-induced oxidative DNA damage *in vivo* in the bone marrow of B6C3F₁ mice. Benzene was administered in corn oil at 880 mg/kg. Control mice were given corn oil alone. a, $P > 0.05$, not significantly different from corresponding corn oil controls; b, $P < 0.001$, significantly different from 1-h corn oil controls; c, $P < 0.05$, significantly different from 3-h corn oil controls.

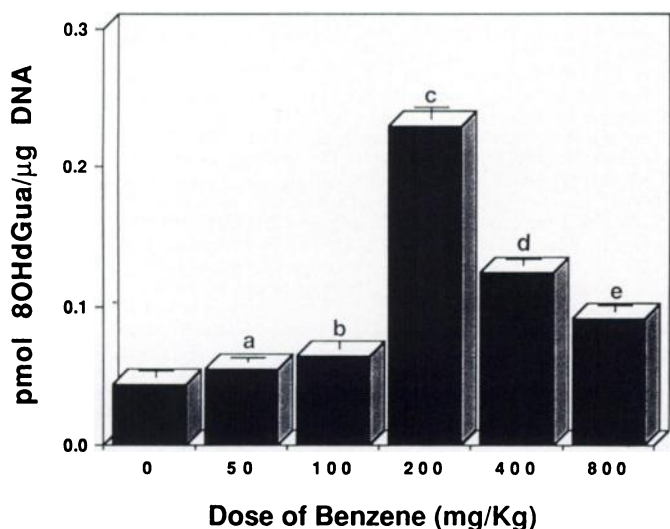


Fig. 2. Dose response for benzene-induced oxidative DNA damage in the bone marrow of B6C3F₁ mice. a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$; d, $P < 0.001$; e, $P < 0.001$; significantly different from corn oil controls.

mg/kg) + CAT (75 mg/kg) was less effective, producing only a slight but significant ($P < 0.01$) increase in the 8OHdGua level. No significant effect on 8OHdGua levels was observed in control mice treated with PBS alone (Fig. 3).

1,2,4-Benzenetriol, a minor but highly reactive phenolic metabolite of benzene, also elicited a significant, 2.5-fold increase ($P < 0.001$) in 8OHdGua levels *in vivo* in mouse bone marrow when administered at 25 mg/kg (Fig. 4). Thus, BT is the only phenolic benzene metabolite which induced oxidative DNA damage in the mouse bone marrow when administered alone. Treatment with BT at 50 and 75 mg/kg resulted in smaller increases in 8OHdGua. This effect is similar to that observed with benzene, where higher doses of benzene resulted in only small increases of 8OHdGua in DNA (Fig. 2).

DISCUSSION

8OHdGua is the most abundant product of oxidative damage to DNA by active oxygen and induces G-T and A-C base substitutions

(29). This suggests that formation of this hydroxylated base may contribute to the mutagenic and carcinogenic properties of radiation and chemicals that generate active oxygen. Peroxidative metabolism or autooxidation of the phenolic metabolites (PH and HQ) of benzene results in active oxygen generation (6, 8, 17). Here, we report that various phenolic metabolites of benzene increase the steady state level of 8OHdGua in the DNA of human leukemia HL60 cells which contains high levels of MPO (23). Further, we have demonstrated that benzene itself and various combinations of its phenolic metabolites produce increases in the steady-state level of 8OHdGua in the bone marrow of B6C3F₁ mice *in vivo*. These results indicate that activation of the phenolic metabolites of benzene (presumably mediated by MPO) produces active oxygen which is capable of causing oxidative DNA damage. We therefore suggest that oxidative damage to DNA may play a role in benzene-induced genotoxicity, myelotoxicity, and leukemia.

Other investigators have previously proposed that oxygen radicals play a important role in benzene toxicity. For example, Anwar *et al.* (30) showed that DMSO, a hydroxyl radical scavenger, inhibits benzene-induced genotoxicity in mice. However, it should be noted that DMSO interacts with hepatic cytochrome P-450 enzymes including

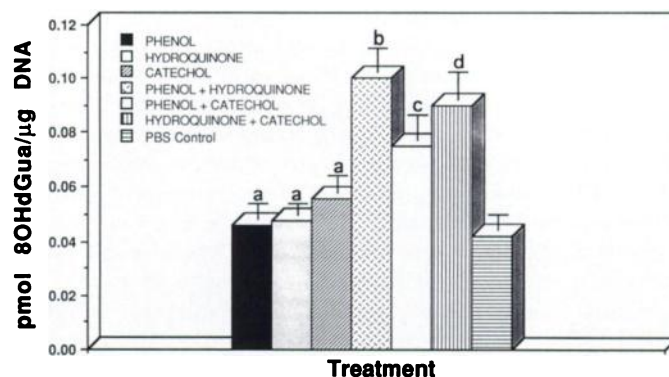


Fig. 3. Induction of oxidative DNA damage by the benzene metabolites, phenol, hydroquinone, and catechol *in vivo* in the bone marrow of B6C3F₁ mice. Metabolites were administered in PBS at 75 mg/kg. Controls received PBS alone. a, $P > 0.05$, not significantly different from PBS controls; b, $P < 0.01$, significantly different from phenol or hydroquinone; c, $P < 0.01$, significantly different from phenol or catechol; d, $P < 0.01$, significantly different from hydroquinone or catechol.

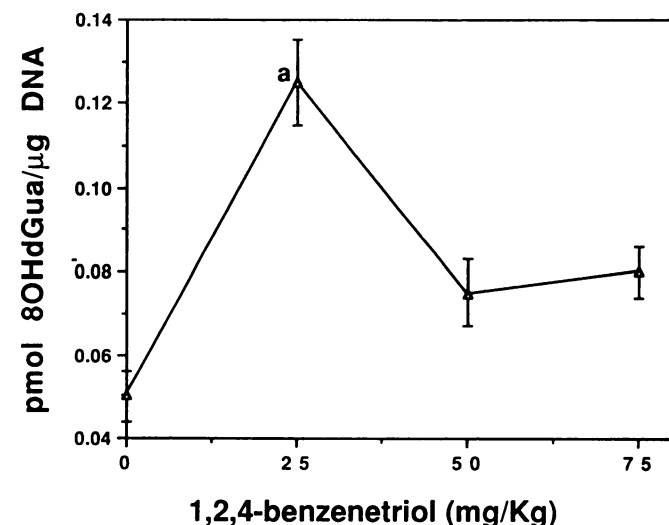


Fig. 4. Induction of oxidative DNA damage *in vivo* in bone marrow of B6C3F₁ mice by 1,2,4-benzenetriol, a minor metabolite of benzene. a, $P < 0.001$, significantly different from PBS controls.

benzene and phenol hydroxylase and cytochrome P450 3a (2E1) (31). Altered metabolism rather than radical scavenging may therefore explain the inhibitory effect of DMSO on the genotoxic effects of benzene. In addition, recent studies by Khan *et al.* (32) and Laskin *et al.* (33) claimed to have shown the production of active oxygen *in vivo* following benzene administration to rats. However, the detection of active oxygen in these studies was performed following *in vitro* stimulation with NADPH/iron (32) and tetradecanoylphorbol acetate (33) (a nonphysiological stimulant) of bone marrow microsomes and macrophages, respectively, isolated from animals exposed to benzene or its phenolic metabolites. Therefore, the data presented in this paper represent the first direct demonstration of active oxygen formation in bone marrow following benzene administration.

Our studies show that the administration of benzene causes a significant increase in 8OHdGua levels in the mouse bone marrow after only 1 h of administration. Interestingly, none of the primary phenolic metabolites of benzene, *i.e.*, PH, HQ, and CAT, were able to induce any change in 8OHdGua levels in the bone marrow when administered alone. However, the administration of BT, a minor phenolic metabolite of benzene, induced a significant increase in 8OHdGua formation in the bone marrow. Interestingly, recent studies by Rao *et al.* (34), have shown that the repeated administration of BT alone to rats for 6 weeks results in myelotoxic effects. BT may, therefore, play some role in benzene toxicity.

There is growing evidence that multiple metabolites play a role in benzene toxicity. For example, Eastmond *et al.* (35) showed that a combination of PH + HQ, each at 75 mg/kg, could reproduce the myelotoxic effects observed following benzene exposure in B6C3F₁ mice. Barale *et al.* (16) have recently shown that the combination of PH and HQ is also highly genotoxic to the mouse bone marrow. Results presented here show that the administration of various combinations of primary phenolic metabolites (at 75 mg/kg each) significantly increases the 8OHdGua level in the mouse bone marrow. PH + HQ was the most effective of the combinations tested, but PH + CAT and CAT + HQ also significantly increased 8OHdGua levels. These results add further weight to the hypothesis that benzene toxicity is caused by multiple metabolites (15, 16, 35). Moreover, since 8OHdGua has been shown to be mutagenic (20, 29) we propose that the genotoxic, myelotoxic, and leukemogenic effects of benzene may be caused at least in part by active oxygen-induced cell damage.

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REFERENCES

- Aksoy, M. Hematotoxicity and carcinogenicity of benzene. *Environ. Health Perspect.*, 82: 193–197, 1989.
- Snyder, R., and Chatterjee, S. P. Benzene metabolism. In: E. Arinik, J. B. Schenkman, and E. Hodgson (eds.), *Proceedings of a NATO Advanced Study Institute on "Molecular Aspects of Monooxygenases and Bioactivation of Toxic Compounds,"* pp. 375–386. New York: Plenum Publishing Corp., 1991.
- Kalf, G. F. Recent advances in the metabolism and toxicity of benzene. *CRC Crit. Rev. Toxicol.*, 18: 141–159, 1987.
- Rickert, D. E., Baker, T. S., Bus, J. S., Barrow, C. S., and Irons, R. D. Benzene disposition in the rat after exposure by inhalation. *Toxicol. Appl. Pharmacol.*, 49: 417–423, 1979.
- Gad-El-Karim, M. M., Ramanujam, V. M., and Legator, M. S. *trans,trans*-Muconic acid, an open-chain urinary metabolite of benzene in mice. Quantitation by high pressure liquid chromatography. *Xenobiotica*, 15: 211–220, 1985.
- Subrahmanyam, V. V., Ross, D., Eastmond, D. A., and Smith, M. T. Potential role of free radicals in benzene-induced myelotoxicity and leukemia. *Free Radical Biol. Med.*, 11: 495–515, 1991.
- Eastmond, D. A., Smith, M. T., Ruvo, L. O., and Ross, D. Metabolic activation of phenol by human myeloperoxidase and horseradish peroxidase. *Mol. Pharmacol.*, 30: 674–679, 1986.
- Sadler, A., Subrahmanyam, V. V., and Ross, D. Oxidation of catechol by horseradish peroxidase and human leukocyte peroxidase: Reactions of *o*-benzoquinone and *o*-benzosemiquinone. *Toxicol. Appl. Pharmacol.*, 98: 62–71, 1988.
- Bhat, R. V., Subrahmanyam, V. V., Sadler, A., and Ross, D. Bioactivation of catechol in rat and human bone marrow cells. *Toxicol. Appl. Pharmacol.*, 94: 297–304, 1988.
- Subrahmanyam, V. V., Kolachana, P., and Smith, M. T. Metabolism of hydroquinone by human myeloperoxidase: mechanisms of stimulation by other phenolic compounds. *Arch. Biochem. Biophys.*, 156: 759–763, 1991.
- Dean, B. J. Recent findings on the genetic toxicology of benzene, toluene, xylenes and phenols. *Mutat. Res.*, 154: 153–181, 1985.
- Yardley-Jones, A., Anderson, D., Lovell, D. P., and Jenkinson, P. C. Analysis of chromosomal aberrations in workers exposed to low level benzene. *Br. J. Indust. Med.*, 47: 48–51, 1990.
- Tice, R., Costa, D., and Drew, R. Cytogenetic effects of inhaled benzene on murine bone marrow: induction of sister chromatid exchanges, chromosomal aberrations and cellular proliferation inhibition in DBA/2 mice. *Proc. Natl. Acad. Sci. USA*, 77: 2148–2155, 1980.
- Yager, J. W., Eastmond, D. A., Robertson, M. L., Paradisin, W. M., and Smith, M. T. Characterization of micronuclei induced in human lymphocytes by benzene metabolites. *Cancer Res.*, 50: 393–399, 1990.
- Robertson, M. L., Eastmond, D. A., and Smith, M. T. Two benzene metabolites, catechol and hydroquinone, produce a synergistic induction of micronuclei and toxicity in cultured human lymphocytes. *Mutat. Res.*, 249: 201–209, 1991.
- Barale, R., Marrazzini, A., Betti, C., Vangelisti, V., Loprieno, N., and Barrai, I. Genotoxicity of two metabolites of benzene: phenol and hydroquinone show strong synergistic effects *in vivo*. *Mutat. Res.*, 244: 15–20, 1990.
- Subrahmanyam, V. V., and O'Brien, P. J. Peroxidase-catalyzed oxygen activation by arylamine carcinogens and phenol. *Chem.-Biol. Interact.*, 56: 185–199, 1985.
- Kasai, H., and Nishimura, S. Hydroxylation of deoxyguanosine at the C-8 position by polyphenols and aminophenols in the presence of hydrogen peroxide and ferric iron. *Jpn. J. Cancer Res.*, 75: 565–566, 1984.
- Floyd, R. A., Watson, J. J., Wong, P. K., Altmiller, D. H., and Rickard, R. C. Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. *Free Radical Res. Commun.*, 1: 163–172, 1986.
- Floyd, R. A. The role of 8-hydroxydeoxyguanosine in carcinogenesis. *Carcinogenesis (Lond.)*, 11: 1447–1450, 1990.
- Kasai, H., Nishimura, S., Kurokawa, Y., and Hayashi, Y. Oral administration of the renal carcinogen, potassium bromate, specifically produces 8-hydroxydeoxyguanosine in rat target organ DNA. *Carcinogenesis (Lond.)*, 8: 1959–1961, 1987.
- Roy, D., Floyd, R. A., and Liehr, J. G. Elevated 8-hydroxydeoxyguanosine levels in DNA of diethylstilbestrol-treated Syrian hamsters: covalent DNA damage by free radicals generated by redox cycling of diethylstilbestrol. *Cancer Res.*, 51: 3882–3885, 1991.
- Yamada, M., Mori, M., and Sugimura, T. Myeloperoxidase in cultured human promyelocytic leukemia cell line HL-60. *Biochem. Biophys. Res. Commun.*, 98: 219–226, 1981.
- Bozeman, P. L., Learn, D. B., and Thomas, E. L. Assay of human leukocyte enzymes myeloperoxidase and eosinophil peroxidase. *J. Immunol. Methods*, 126: 125–133, 1990.
- Gupta, R. C. Nonrandom binding of the carcinogen *N*-hydroxy-2-acetylaminofluorene to repetitive sequences of rat liver DNA *in vivo*. *Proc. Natl. Acad. Sci. USA*, 81: 6943–6947, 1984.
- Park, E. M., Shigenaga, M. K., Degan, P., Korn, T. S., Kitzler, J. W., Wehr, C. M., Kolachana, P., and Ames, B. N. Assay of excised oxidative DNA lesions: isolation of 8-oxoguanine and its nucleoside derivatives from biological fluids with a monoclonal antibody column. *Proc. Natl. Acad. Sci. USA*, 89: 3375–3379, 1992.
- Kasai, H., and Nishimura, S. Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Res.*, 12: 2137–2145, 1984.
- Lee, E. W., Kocsis, J. J., and Snyder, R. Acute effect of ⁵⁹Fe incorporation into circulating erythrocytes. *Toxicol. Appl. Pharmacol.*, 27: 431–436, 1974.
- Cheng, K. C., Cahill, D. S., Kasai, H., Nishimura, S., and Loeb, L. A. 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G-T and A-C substitutions. *J. Biol. Chem.*, 267: 167–172, 1992.
- Anwar, W. A., Au, W. W., Legator, M. S., and Ramanujam, V. M. S. Effect of dimethylsulfoxide on the genotoxicity and metabolism of benzene *in vivo*. *Carcinogenesis (Lond.)*, 10: 441–445, 1989.
- Morgan, E. T., Koop, D. R., and Coon, M. J. Catalytic activity of cytochrome P-450 isozyme 3a isolated from liver microsomes of ethanol-treated rabbits: oxidation of alcohols. *J. Biol. Chem.*, 257: 13951–13957, 1982.
- Khan, S., Krishnamurthy, R., and Pandya, K. P. Generation of hydroxyl radicals during benzene toxicity. *Biochem. Pharmacol.*, 33: 2009–2112, 1990.
- Laskin, D. L., MacEashern, L., and Snyder, R. Activation of phagocytes following benzene treatment of mice. *Environ. Health Perspect.*, 82: 75–79, 1989.
- Rao, G. S., Siddiqui, S. M., Pandya, K. P., and Shanker, R. Relative toxicity of metabolites of benzene in mice. *Vet. Human Toxicol.*, 30: 517–520, 1988.
- Eastmond, D. A., Smith, M. T., and Irons, R. D. An interaction of benzene metabolites reproduces the myelotoxicity associated with benzene exposure. *Toxicol. Appl. Pharmacol.*, 91: 85–95, 1987.