An Overview of Benzene Metabolism

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Benzene toxicity involves both bone marrow depression and leukemogenesis caused by damage to multiple classes of hematopoietic cells and a variety of hematopoietic cell functions. Study of the relationship between the metabolism and toxicity of benzene indicates that several metabolites of benzene play significant roles in generating benzene toxicity. Benzene is metabolized, primarily in the liver, to a variety of hydroxylated and ring-opened products that are transported to the bone marrow where subsequent secondary metabolism occurs. Two potential mechanisms by which benzene metabolites may damage cellular macromolecules to induce toxicity include the covalent binding of reactive metabolites of benzene and the capacity of benzene metabolites to induce oxidative damage. Although the relative contributions of each of these mechanisms to toxicity remains unestablished, it is clear that different mechanisms contribute to the toxicities associated with different metabolites. As a corollary, it is unlikely that benzene toxicity can be described as the result of the interaction of a single metabolite with a single biological target. Continued investigation of the metabolism of benzene and its metabolites will allow us to determine the specific combination of metabolites as well as the biological target(s) involved in toxicity and will ultimately lead to our understanding of the relationship between the production of benzene metabolites and bone marrow toxicity. — Environ Health Perspect 104(Suppl 6):1165–1171 (1996)

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Introduction

The identification of benzene metabolites and their quantification began in the 19th century. However, it was not until carbon-14 was available for the synthesis of [^{14}C]benzene that it was possible to perform accurate measurements and to be sure that all the stable metabolites could be detected. Thus, in 1953 Parke and Williams (*1*) reported that upon administering [^{14}C]benzene to rabbits they could recover 32.6% of the dose in urine as phenol, catechol, hydroquinone, 1,2,4-benzenetriol, *trans-trans*-muconic acid, and L-phenylmercapturic acid, 44.5% in the expired air as unchanged benzene and small amounts of carbon dioxide, and 5 to 10% in the feces and tissues. Their total recovery was approximately 84 to 89%. They went on to suggest that benzene toxicity, i.e., benzene-induced bone marrow depression, might be caused by some of these metabolites.

Review of Benzene Toxicity

A discussion of the role of benzene metabolites in benzene toxicity requires a brief review of benzene toxicity (2,3). Benzene toxicity to humans exposed in the workplace has been characterized as either early reversible hematotoxicity or, with prolonged exposure to high doses, irreversible bone marrow damage. Studies of worker populations in factories in which benzene was employed as a solvent (*2*) showed a range of hematotoxic effects including anemia, leukopenia, and thrombocytopenia. In some cases, more than one cell type was decreased. A decrease in the levels of all the classes of blood cell types in the circulation is termed pancytopenia and is usually associated with irreversible bone marrow aplasia. Aplastic anemia is in most cases fatal. In those who survive aplasia, the marrow appears to be dysplastic. Myelodysplastic syndrome, which has been called "preleukemia," is probably an early stage of acute myeloid leukemia. Thus, those interested in benzene metabolism need to determine how benzene metabolites contribute to the production of this series of events associated with toxicity.

Benzene Metabolism and Toxicity

The evidence is quite strong that benzene metabolism plays a critical role in benzene toxicity (*2,3*). Thus, inhibition of benzene metabolism by toluene, a competitive inhibitor, results in a decrease in benzene metabolism and a reduction in benzene toxicity (*4*). Decreasing the hepatic metabolism of benzene by partial hepatectomy also reduced benzene toxicity, suggesting that hepatic metabolism plays an important role in toxicity (*5*). In addition to hepatic metabolism, it appears that secondary metabolism of benzene metabolites in bone marrow contributes to toxicity (*6–11*). Thus, elucidation of the metabolic pathway for benzene biotransformation is essential for a full understanding of the mechanism of toxicity.

Metabolic Pathway for Biotransformation

Figures 1 and 2 show alternative routes by which the first step of benzene metabolism, namely, phenol formation, can occur. Cytochrome P4502E1, and perhaps other cytochromes P450, can generate H_2O_2 when acting as oxidases of nicotinamide adenine dinucleotide phosphate (NADPH). The hydroxyl radical formed from H_2O_2 can hydroxylate benzene to yield phenol. An alternative mechanism for phenol formation is seen in Figure 2, which is designed to reflect on the fate of the benzene oxide–oxepin system. When benzene oxide is the first product, it can rearrange nonenzymatically to form phenol. Alternatively, benzene oxide can be hydrated via epoxide hydrolase to yield 1,2-benzene dihydrodiol, which can in turn
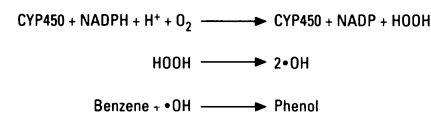
$$CYP450 + NADPH + H^+ + O_2 \longrightarrow CYP450 + NADP + HOOH$$

$$HOOH \longrightarrow 2 \bullet OH$$

$$Benzene + \bullet OH \longrightarrow Phenol$$

Figure 1. An alternative route of phenol formation from benzene: hydroxylation of benzene by hydroxyl radicals generated from H_2O_2.

be oxidized via dihydrodiol dehydrogenase to form catechol. The reaction of benzene oxide with glutathione catalyzed by glutathione S-transferase leads to the formation of the premercapturic acid. It is likely that benzene oxide or its oxepin are precursors to ring opening (12). Phenol can be further hydroxylated to form hydroquinone or catechol. In theory, 1,2,4-benzenetriol may be formed by the hydroxylation of either hydroquinone or catechol; but Inoue et al. (13) suggested that catechol is not a precursor of 1,2,4-benzenetriol in humans.

Urinary Metabolites

The metabolites of benzene found in urine are shown in Figure 3. Any of the phenolic metabolites may be conjugated with either sulfate or glucuronide. In addition to L-phenylmercapturic acid reported by Parke and Williams (1), other mercapturates include 6-N-acetylcysteinyl-S-2,3-cyclohexadienol (14,15) and 2,5-diOH-phenylmercapturic acid (16). The urine also contains two ring-opening products, i.e., trans-trans-muconic acid (1,17) and 6-OH-t,t-2,4-hexadienoic acid (18), and the residue of a covalently bound DNA adduct, i.e., N^7-phenylguanine (19,20).

Microsomal Metabolism

The metabolism of benzene by purified, reconstituted rat liver CYP4502E1 is characterized by the requirement of the presence of cytochrome b_5 to obtain maximum metabolism (21). At low benzene concentrations (Table 1), a much larger percentage of hydroquinone is formed than at higher benzene concentrations. The addition of epoxide hydrolase also stimulates hydroquinone formation at the expense of phenol. The role of epoxide hydrolase in stimulating the second hydroxylation of benzene is not clear. It may assist in stabilizing CYP4502E1 to continue to hydroxylate phenol, the concentration of which rises as benzene metabolism proceeds. Alternatively, it may reflect a hitherto unrecognized metabolic activity leading to hydroquinone formation. Further study of this problem is required.

Potential Mechanisms of Toxicity

The production of benzene metabolites, largely in the liver, is followed by their transport to the bone marrow and other organs. There are many possibilities for causing bone marrow toxicity. Irons and coworkers (22,23) suggested that covalent binding of hydroquinone to spindle fiber protein could explain inhibition of cell
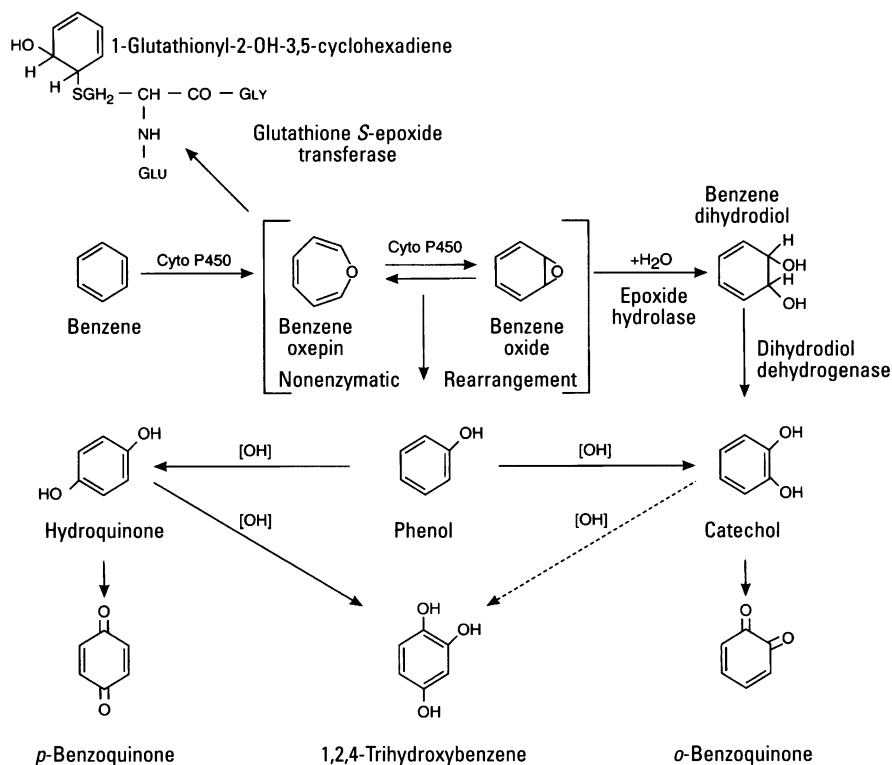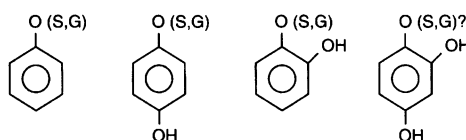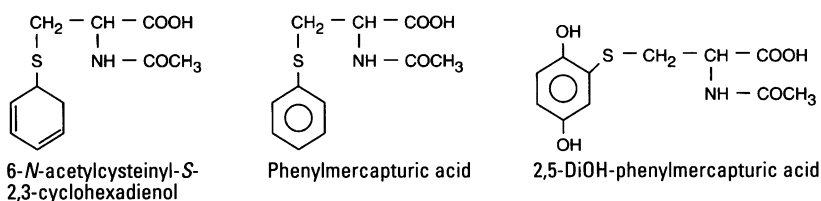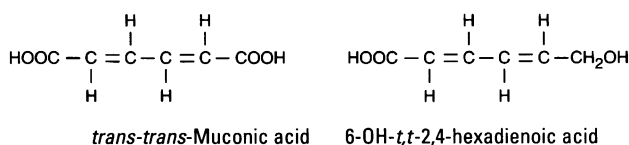

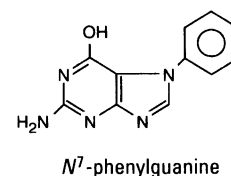**Figure 2.** The metabolic fate of the benzene oxide–oxepin system.

Figure 3. The urinary metabolites of benzene.

replication by benzene. Damage to DNA could result in bone marrow depression leading to aplastic anemia, which in survivors leads to marrow dysplasia and ultimately to acute myeloid leukemia (24). Figure 4 diagrams two mechanisms by which benzene metabolites could cause damage to DNA. One pathway focuses on

Table 1. Benzene metabolism by purified reconstituted cytochrome P4502E1 and its modulation by cytochrome b_5 and microsomal epoxide hydrolase.[a]

Benzene, mM	Phenol nmol/min/ μmol P4502E1	Hydroquinone nmol/ min/μmol P4502E1	Total
Without cytochrome b_5			
0.02	0.16 ± 0.02 (84)	0.03 ± 0.01 (16)	0.19 ± 0.01
0.08	0.46 ± 0.08 (92)	0.04 ± 0.02 (8)	0.50 ± 0.11
0.80	2.40 ± 0.02 (94)	0.15 ± 0.03 (6)	2.55 ± 0.23
4.0	5.28 ± 0.12 (95)	0.29 ± 0.03 (5)	5.57 ± 0.10
With cytochrome b_5			
0.02	0.7 ± 0.01 (60)	0.46 ± 0.01 (40)	1.16 ± 0.01
0.08	2.04 ± 0.11 (72)	0.77 ± 0.07 (28)	2.85 ± 0.14
0.8	7.45 ± 0.13 (91)	0.76 ± 0.04 (9)	8.21 ± 0.20
4.0	9.84 ± 0.20 (95)	0.50 ± 0.02 (5)	10.34 ± 0.23
Metabolism of 0.8 mM benzene in the presence of microsomal epoxide hydrolase			
Units EH[b]	Phenol	Hydroquinone	Total
0	7.45 ± 0.13	0.76 ± 0.04	8.21 ± 0.09
16	7.54 ± 0.16	0.83 ± 0.05	8.37 ± 0.18
32	8.40 ± 0.94	1.54 ± 0.13	10.12 ± 0.83
64	8.74 ± 0.37	3.19 ± 0.50	12.35 ± 0.82

[a]Values represent the mean ± standard deviation. Percentages of total metabolism resulting in either phenol or hydroquinone formation are shown in parentheses. [b]Units EH = amount of EH producing 1 μmol of styrene glycol from styrene oxide per minute; EH was added subsequent to b_5 addition and prior to combination with lipid.

Figure 4. Potential pathways of DNA damage by benzene in bone marrow cells.

Table 2. Water-soluble and covalently bound metabolites of [³H]benzene in organs of CD-1 mice given 2 doses per day (880 mg/kg) for 3 days.[a]

Organ	Water-soluble metabolites[b]	Convalently bound metabolites[c]
Bone marrow	2615 ± 124	62 ± 18
Liver	1377 ± 217	669 ± 188
Kidney	1306 ± 217	873 ± 101
Lung	1130 ± 145	223 ± 20
Spleen	774 ± 76	176 ± 12
Blood	1161 ± 199	183 ± 35
Muscle	1076 ± 411	60 ± 11

[a]Mice were sacrificed 12 to 16 hr after the last dose. [b]Expressed as nmol benzene equivalents per gram wet weight. [c]Expressed as nmol benzene equivalents per gram protein.

Figure 5. Structures of the major deoxyribonucleoside adducts of p-benzoquinone (A) 3'-OH-1,N^2-benzetheno-2'-deoxyguanosine; (B) 3'-OH-3,N^4-benzetheno-2'-deoxycytidine; (C) 3'-OH-1,N^6-benzetheno-2'-deoxyadenosine (dR = 2–deoxyribose).
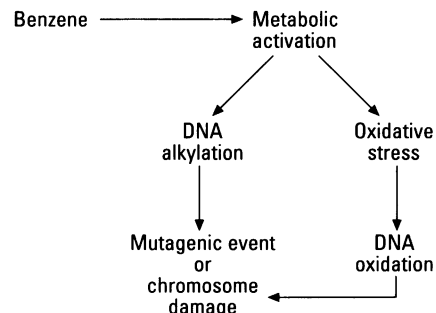
the metabolic activation of benzene to species that covalently bind to DNA to produce mutagenic events that are expressed as leukemia. The second mechanism involves the production of metabolites that cause oxidative stress, subsequent oxidative damage to DNA, and a mutagenic effect that has the same consequences.

Covalent Binding and Toxicity

We have known for almost two decades that benzene is metabolized to species capable of covalent interaction with cellular macromolecules. Snyder et al. (25) first demonstrated that proteins in the bone marrow and other tissues of mice treated with radiolabeled benzene *in vivo* contained covalently bound radiolabel (Table 2). Lutz and Schlatter subsequently demonstrated covalent binding of benzene metabolites to DNA in rat liver (26). Our studies of the covalent interaction of benzene metabolites with cellular macromolecules suggested that this phenomenon might play an important role in the expression of toxicity. Sammett et al. (5) showed that in rats partial hepatectomy correlated with both protection against benzene toxicity and reduced levels of covalent binding of benzene metabolites in bone marrow; Longacre et al. (27) showed that the levels of covalently bound metabolites measured in the hematopoietic tissues were higher in mouse strains that were more sensitive to benzene toxicity than in those that were less sensitive. Rushmore et al. (28) extensively investigated covalent binding in an isolated

mitochondrial system; they showed that the benzene metabolites are capable of covalent binding to DNA and inhibiting protein and RNA synthesis. To chemically characterize the DNA adducts formed, adducts were prepared *in vitro* by reacting deoxynucleosides or deoxynucleoside monophosphates with either p-benzoquinone or hydroquinone in the presence of an oxidizing agent. The combination of UV, fluorescence, mass, and nuclear magnetic resonance spectrometry was first used by Jowa et al. (29) to identify 3'-OH-1,N^2-benzetheno-2'-deoxyguanosine as a major deoxyguanosine adduct (Figure 5). Pongracz et al. (30,31) and Levay et al. (32) subsequently combined these spectroscopic methods of structural analysis with the sensitive DNA [³²P]postlabeling method of adduct detection to identify (3'-OH)-3,N^4-benzethenodeoxycytidine-3'–phosphate, (3'-OH)-1,N^6-benzetheno-2'-deoxyadenosine-3'-phosphate and (3'-OH)-1,N^2-benzetheno-2'deoxyguanosine-3'-phosphate following the *in vitro* reaction of p-benzoquinone and calf thymus DNA. The structures of the deoxyribonucleoside forms of these adducts are shown in Figure 5.
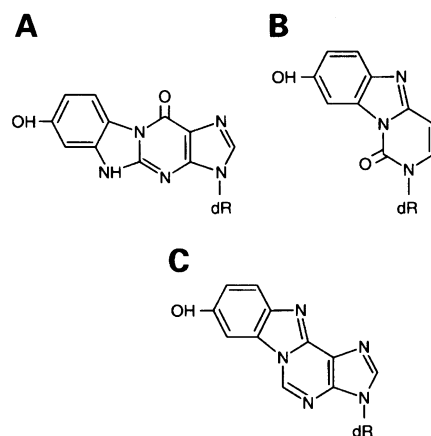
Although *in vitro* studies have established that reactive metabolites of benzene covalently bind to DNA, *in vivo* evidence of covalent binding has been more difficult to demonstrate. Initial studies involving administration of radiolabeled benzene to rats by Lutz and Schlatter (26) as well as later studies by Arfellini et al. (33) and Mazullo et al. (34) using rats and mice,

demonstrated covalent binding to DNA of several animal tissues including liver, bone marrow, spleen, kidney, stomach, and lung. Attempts to identify adduct formation *in vivo* using the [³²P]postlabeling method have not, however, been highly successful.

These problems may be related to the low covalent binding index of benzene, the complex nature of the bone marrow, and difficulties in establishing both an optimal treatment regimen and an animal model system that accurately reflects all the toxic responses to benzene observed in humans.

The most consistent demonstrations of benzene metabolite-induced DNA adduct formation in a cellular model have been made using human promyelocytic (HL-60) cells in culture, a line of myeloid cells that has the capacity to differentiate in response to specific chemical stimulants into any of the four classes of hematopoietic cells of the myelomonocytic lineage, i.e., granulocytes, monocytes, eosinophils, or macrophages (35). Studies using this model and the [32P]postlabeling method of adduct detection by Levay et al. have led to the detection of DNA adducts in benzene metabolite-treated cells that are not chromatographically identical with those formed following the in vitro reaction of p-benzoquinone and DNA (32). Although these investigations showed that benzene metabolites interact synergistically to produce DNA adducts (36) and that peroxidase activation of hydroquinone is required for adduct formation (37), no attempts were made to link adduct formation with any end point of toxicity other than cytotoxicity. We recently evaluated the significance of DNA adduct formation in toxicity by studying the effects of benzene metabolites on DNA adduct formation and retinoic acid-induced granulocytic differentiation in this model (38). Table 3 shows that while treatment of HL-60 cells with 50 μM hydroquinone for 1 to 4 hr induced the formation of a single DNA adduct that increased with increasing time of exposure, no adducts were detected in cells treated with 50 to 500 μM 1,2,4-benzenetriol for up to 4 hr. Using the same incubation conditions, treatment of the cells with either hydroquinone or 1,2,4-benzenetriol prior to inducing differentiation with retinoic acid significantly inhibited their

capacity to differentiate, as assessed by evaluating cell morphology, using light and electron microscopy, and two indicators of cell functional capacity—phagocytosis and nitroblue tetrazolium reduction. These data indicate that DNA adduct formation may play a role in inhibiting cell differentiation in hydroquinone but not in 1,2,4-benzenetriol-treated cells, and support the contention that various metabolites contribute to different components of the mechanism of toxicity.

Oxidative Stress and Toxicity

The potential for oxidative stress to contribute to benzene toxicity is closely tied to specific benzene metabolites. Hydroquinone may be oxidized to p-benzoquinone, which is highly reactive and can covalently bind to cellular macromolecules (above) or to glutathione. Alternatively,

benzene metabolites may engage in redox cycling, which involves autooxidation of a reduced form of the metabolite to yield an oxidized species plus reactive oxygen. The bone marrow, which is a richly oxygenated organ, has the capability to generate reactive oxygen species. The four-electron reduction of oxygen (39) may generate superoxide anion radical, hydrogen peroxide, and hydroxyl radical. The oxidized metabolite may undergo flavoprotein reduction to yield the starting material that may reenter the redox cycle.

It might be postulated that hydroquinone–p-benzoquinone would be likely to undergo redox cycling. Recent studies by Boersma et al. (40) argue to the contrary. Figure 6 shows that the reduction of p-benzoquinone may proceed via a reductase such as CYP450 reductase in two steps. The first product would be the semiquinone anion
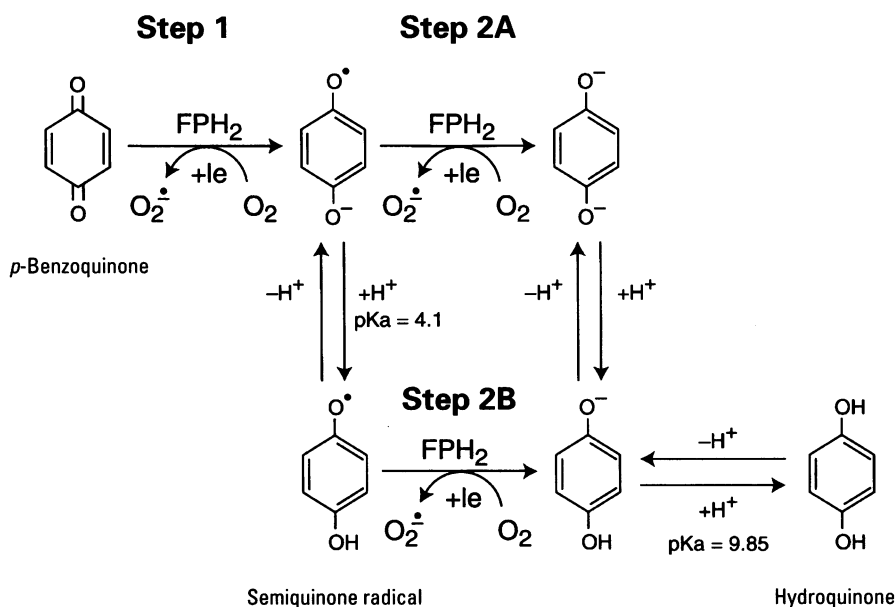
Figure 6. Two-step reduction of p-benzoquinone by reductase (FPH2): evidence that p-benzoquinone does not support redox cycling at physiological pH. Steps 1, 2A, and 2B are sites at which reactions with oxygen to yield superoxide would occur in redox cycling. However, p-benzoquinone is stoichiometrically reduced to hydroquinone at pH 7.5, the pH of the cell (40).

Table 3. Effect of benzene metabolite treatment on granlocytic differentiation and DNA adduct formation in HL-60 cells.

Treatment	DNA binding by [32P]postlabeling	Effect of indicated treatments on retinoic acid-induced cell differentiation		
		Morphological evaluation by light and electron microscopy	Functional evaluation	
			Phagocytosis of sheep red blood cells	NBT reduction
Negative-control untreated cells	None	Immature undifferentiated	−	−
Positive-control untreated—RA induced	Not analyzed	Mature undifferentiated	+	+
50 μM HQ/0.5 to 4 hr	Single adduct/time-dependent increase	Less mature cells, differentiation inhibited	↓	↓
50 μM BT/2 hr	Not detectable	Differentiation inhibited	↓	↓
250 μM BT/ 2 hr, 4 hr	Not detectable	Diffentiation inhibited	↓	↓
500 μM BT/4 hr	Not detectable	Not analyzed	Not analyzed	Not analyzed

radical (Step 1), which could either be reduced again (Step 2A), or may—more likely—be protonated before the second reduction (Step 2B). The pK_a of the protonation step is 4.1, suggesting that at the pH of the cell, i.e., approximately 7.5, theanionic form would predominate. Following the second reduction, the monoanion of hydroquinone would be formed, but the pK_a for its protonation is 9.85, indicating that it would exist mainly in the diprotonated form. Reoxidation to p-benzoquinone, the next step in redox cycling, would be inhibited because it is the monanion that is the substrate for autooxidation leading to superoxide anion formation. Thus, it is unlikely that hydroquinone-p-benzoquinone undergoes redox cycling at physiological pH.

The metabolic fate of p-benzoquinone, if it is not reduced, may be to react with glutathione (GSH) to form the premercapturic acid of hydroquinone, which may go on to form the mercapturic acid, or may undergo slow autooxidation leading to the production of reactive oxygen species (41) (Figure 7). Alternatively, p-benzoquinone may be converted to its epoxide either via CYP450 or H00H (Figure 8, Step 1), leading to p-benzoquinone 2,3-oxide and ultimately to 1,2,4-benzenetriol (Step 2A) following either a two-electron reduction by diaphorase or two one-electron reductions by CYP450 reductase. Reaction of p-benzoquinone 2,3-oxide with GSH leads to the formation of glutathionyl 1,2,4-benzenetriol (Figure 8, Step 2B).

The unique feature of glutathionyl 1,2,4-benzenetriol is its ability to spontaneously undergo autooxidation and redox cycling (Figure 9). Brunmark and Cadenas (41) showed that glutathionyl benzenetriol could be sequentially oxidized to glutathionyl-1,2,4-benzenetriol semiquinone
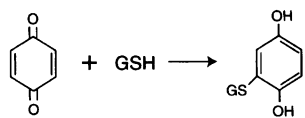
and then to 5-OH-glutathionyl-p-benzoquinone (HGB). HGB is also the product of the reaction between p-benzoquinone 2,3-oxide and glutathionyl-1,2,4-benzenetriol semiquinone (GBS), which also yields 5-OH-benzosemiquinone. Alternatively, GBS can undergo disproportionation to form glutathionyl-1,2,4-benzenetriol, which can initiate the redox cycle again. If redox cycling is an important phenomenon in the mechanism of benzene toxicity, it is likely that 1,2,4-benzenetriol plays a significant role in the process.

In a series of studies in HL-60 cells, hydroquinone, p-benzoquinone and 1,2,4-benzenetriol were added so that researchers could study their impacts on oxidative stress and antioxidant factors (42). Table 4 shows that hydroquinone and p-benzoquinone increased superoxide, nitric oxide, and HOOH production, but that 1,2,4-benzenetriol, while increasing superoxide and HOOH, had no effect on nitric oxide production. Hydroquinone and p-benzoquinone but not 1,2,4-benzenetriol decreased catalase activity. Hydroquinone and 1,2,4-benzenetriol but not p-benzoquinone decreased superoxide dismutase and hydroquinone and benzenetriol decreased sulfhydryl levels. Thus, although all of the metabolites induce oxidative

Figure 8. The formation of p-benzoquinone 2,3-oxide from p-benzoquinone and its metabolic fate. Step 1. p-Benzoquinone is converted to its epoxide by the addition of HOOH. Step 2A. p-Benzoquinone is reduced to 1,2,4-benzenetriol by either one-electron reduction (1) catalyzed by P450 reductase or two-electron reduction (2) catalyzed by DT diaphorase. Step 2B. Reaction of 1,2,4-benzenetriol with glutathione to yield glutathionyl 1,2,4-benzenetriol.

Figure 9. The redox cycling of glutathionyl benzenetriol. 1. Formation of 2-OH-5-glutathionyl p-benzoquinone from 2-OH-glutathionyl hydroquinone by autooxidation. 2. Formation of 2-OH-5-glutathionyl p-benzoquinone via redox transition of the hydroxysemiquinone and p-benzoquinone 2,3-oxide. 3. Formation of 2-OH-5-glutathionyl p-benzoquinone via disproportionation of the hydroxysemiquinone.
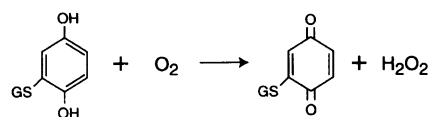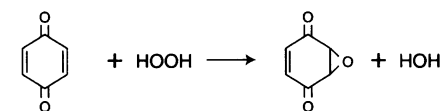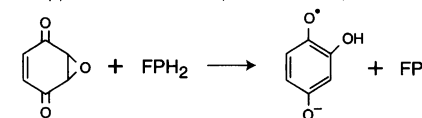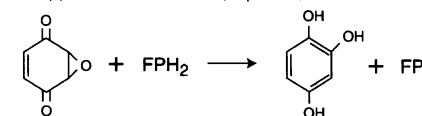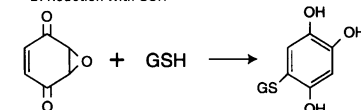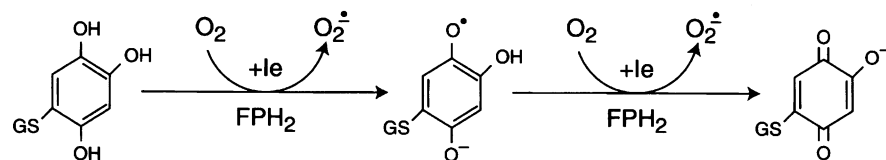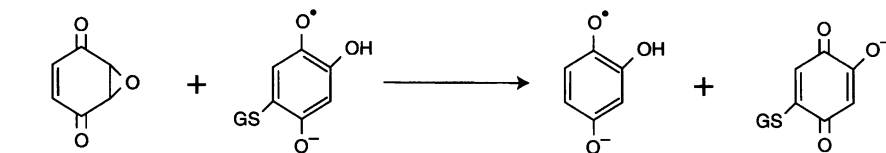

Figure 7. Potential interaction of p-benzoquinone with glutathione (1) anaerobic (1:1 stoichiometry; rapid); (2) aerobic (slow autooxidation).

Table 4. Impact of hydroquinone, p-benzoquinone, and 1,2,4-benzenetriol on oxidative stress and antioxidant factors in HL-60 cells activated by 12-O-tetradecanoylphorbol-13-acetate.

Factor	HQ	BQ	BT	Method
Superoxide	Increase	Increase	Increase	Reid and Loeb (45)
Nitric oxide	Increase	Increase	No change	Green et al. (46)
Hydrogen peroxide	Increase	Increase	Increase	Bass et al. (47)
Myeloperoxidase	No change	No change	No change	Himmelhoch et al. (48)
Catalase	Decrease	Decrease	No change	Aebi (49)
SOD	Decrease	No change	Decrease	Marklund and Marklund (50)
Vitamin C	No change	No change	No change	Attwood et al. (51)
Thiols (GSH)	Decrease	No change	Decrease	Beutler et al. (52)

Table 5. Relative potencies of benzene and benzene metabolites in short-term tests of erythropoiesis.[a]

Compound	Dose, mg/kg (3 doses, 2 days)
Benzene	1000
Phenol	–
Hydroquinone sulfate	300
Hydroquinone	100
Catechol	100
6-OH-t,t-2, 4-hexadienal (CHO-M-OH)	20
Hydroquinone + phenol	10
Catechol + phenol	10
1,2,3-Benzenetriol	–
p-Benzoquinone	1
Muconaldehyde	1
Muconaldehyde + hydroquinone	0.05–0.01 (est)[b]

[a]Measured using the [^{59}Fe] uptake assay (43).
[b]Estimated value.

modifications in these cells, their individual impacts on oxidative stress and antioxidant factors in these cells are different.

Potency of Benzene and Its Metabolites in Producing Toxicity

In attempting to sort out the role played by the various benzene metabolites in the production of benzene toxicity, it is helpful to examine the potency with which each of the metabolites, alone or in combination, causes toxic effects. There are many stages in bone marrow cell maturation and amplification and there are a number of functions peculiar to the stromal cells that provide targets for attack by benzene and its metabolites. It would be helpful to review a range of potencies of each. Table 5 shows the relative potency with which benzene and its metabolites inhibit erythropoiesis, as measured by the method of Lee et al. (43) using the [^{59}Fe] uptake technique. The numbers are not absolute values but are rounded off to demonstrate the range of doses at which significant

depressions in red cell production were observed. The data demonstrate that when administered to mice in a defined dosing regimen, benzene is the least potent member of the series and the combination of hydroquinone plus muconaldehyde provides the greatest potency. The doses of each chemical used in the latter case were too low for either muconaldehyde or hydroquinone to produce bone marrow depression given independently, but they were highly effective when given in combination. Many of the other benzene metabolites were effective at decreasing iron uptake, albeit with different potencies, except for phenol, which was clearly ineffective. 1,2,4-Benzenetriol was ineffective in preliminary studies, but further work is needed to establish the significance of these observations, since a decrease in lymphocytes has been observed in the bone marrow of animals treated with 1,2,4-benzenetriol (CC Hedli and R Snyder, unpublished observations). In addition, the recent demonstration of 1,2,4-benzenetriol as a microsomal metabolite of benzene in isolated mouse but not rat hepatocytes (44) suggests that the production of

this metabolite might correlate with susceptibility to toxicity. Further experiments are in progress to determine the effect of 1,2,4-benzenetriol treatment on circulating blood cells and other hematopoietic tissue.

Summary and Conclusions

Benzene toxicity, which involves both bone marrow depression and leukemogenesis, appears to require metabolites of benzene that impinge on several cell types and on a variety of functions. We must continue to study the metabolism of benzene with the intent of understanding which bone marrow cells metabolize benzene and its metabolites, identifying the specific array of metabolites responsible for the disease processes, and ultimately understanding the relationship between the events in bone marrow toxicity and the generation of specific metabolites.

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