Analysis of Target Cell Susceptibility as a Basis for the Development of a Chemoprotective Strategy against Benzene-induced Hematotoxicities

Michael A. Trush, Lorraine E. Twerdok,* Stephen J. Rembish,† Hong Zhu, and Yunbo Li

Division of Toxicological Sciences, Department of Environmental Health Sciences, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland

A goal of our research is to identify biochemical factors that underlie the susceptibility of bone marrow cell populations to benzene metabolites so as to develop a mechanistically based chemoprotective strategy that may be used in susceptible humans exposed to benzene. By doing biochemical risk analysis of bone marrow stromal cells from mice and rats and the human myeloid cell lines, HL-60 and ML-1; and by using buthionine sulfoximine and dicumarol we have observed that the susceptibility of these cell populations to hydroquinone (HQ) correlates with their concentration of glutathione (GSH) and activity of quinone reductase (QR). Accordingly, the induction of QR and GSH by 1,2-dithiole-3-thione (D3T) in these cell populations has resulted in a significant protection against the following hydroquinone-mediated toxicities: inhibition of cell proliferation and viability; reduced ability of stromal cells to support myelopoiesis; and altered differentiation of ML-1 cells to monocytes/macrophages. Preliminary *in vivo* experiments indicate that feeding mice D3T results in an induction of QR in the bone marrow compartment such that stromal cells are more resistant to hydroquinone-induced cytotoxicity *in vitro*. Overall, these studies suggest that in addition to hepatic cytochrome P4502E1, bone marrow QR and GSH are factors that could determine an individual's relative susceptibility to the toxic effects of benzene. — Environ Health Perspect 104(Suppl 6):1227–1234 (1996)

Key words: bone marrow stromal cells, HL-60, ML-1, chemoprotection, quinone reductase, glutathione, susceptibility, 1,2-dithiole-3-thione, hydroquinone

Introduction

Although physicians have been aware of hematotoxicity resulting from benzene exposure for nearly a century (*1*), there is still concern for human health due to continued benzene exposure in certain occupational settings and as a result of cigarette smoking (*2*). One of the goals of toxicology as a science is to develop preventive interventions against chemical-induced disease. The development of rational chemoprotective strategies can result from an understanding of the underlying mechanisms involved in the induction of disease by a particular chemical (Figure 1). Such mechanistically based chemoprotective interventions can then be utilized in populations that cannot avoid exposure to a chemical or in exposed individuals within a population who may be inherently susceptible to even low concentrations of a chemical. The present studies in China examining oltipraz as a chemoprotective agent against aflatoxin-induced hepatocarcinogenesis are an example of this approach (*3*).

As early as the 1920s occupational health physicians recognized that there are individual differences in susceptibility to benzene-induced hematotoxicity (*1,4*). As with many chemicals, it is now appreciated that the susceptibility to benzene is determined in part by the activity of enzymes involved in its biotransformation, as well as by factors involved in the detoxification of benzene-derived reactive intermediates. Although human exposure to benzene occurs primarily through inhalation, biotransformation reactions in the liver result in phase I and phase II metabolites (*5*). Subsequent bioactivation of some of these liver-derived metabolites within the various bone marrow cell populations results in altered bone marrow function leading to the manifestations of benzene-induced hematotoxicity: aplastic anemia; leukemia; and immunotoxicity (*2*). In the liver, the metabolism of benzene to the phase I metabolites, phenol, and hydroquinone (HQ), is catalyzed by cytochrome P4502E1 (CYP2E1) (*6*). Since phenol and HQ can be further converted to reactive intermediates, the activity of CYP2E1 in the liver can be viewed as a factor that contributes to individual susceptibility (*7*). Likewise, within the bone marrow there are factors that probably determine the susceptibility of the various bone marrow cell populations to toxic reactions by benzene-derived metabolites. For example, the bioreactivity of benzoquinone, an electrophilic metabolite of HQ, can be modulated by the actions of quinone reductase (QR) or glutathione (GSH) (Figure 2). The purpose of this report is to illustrate why QR and GSH should be considered determinants of HQ-induced toxicity to bone marrow cells and to demonstrate that the induction of QR and GSH may serve as a basis for the development of a chemoprotective strategy against benzene-induced hematoxicities.

Determinants of Toxicity to Bone Marrow Cell Populations

Within the bone marrow are a number of cell populations that can serve as potential targets of environmental chemicals or drugs (Figure 3). These include the hemopoietic and lymphopoietic stem cells, committed progenitors, immature hemopoietic precursors, mature functional blood cells,

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Address correspondence to Dr. M.A. Trush, Johns Hopkins School of Hygiene and Public Health, 615 N. Wolfe St., Room 7032, Baltimore, MD 21205. Telephone: (410) 955-4712. Fax: (410) 955-0116. E-mail: mtrush@phnet.sph.jhu.edu

*Present address: American Petroleum Institute, Washington, DC.

†Present address: Texas Natural Resource Commission, Austin, TX.

Abbreviations used: B[*a*]P, benzo[*a*]pyrene; *t*-BHQ, *tert*-butylhydroquinone; BSO, buthionine sulfoximine; CYP2E1, cytochrome P4502E1; DMSO, dimethyl sulfoxide; D3T, 1,2-dithiole-3-thione; GSH, glutathione; HQ, hydroquinone; IL-1, interleukin-1; QR, quinone reductase; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

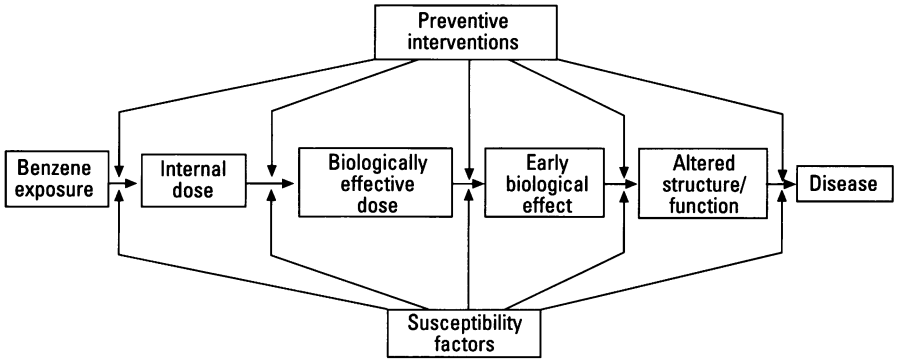

Figure 1. Steps in the induction of disease following benzene exposure.

and the various cells that comprise the bone marrow stromal microenvironment (*8*). As shown in Figure 3, the altered function of different populations probably results in the manifestation of different types of toxicities. For example, inhibition of the ability of stromal macrophages to synthesize interleukin-1 (IL-1) results in the altered differentiation of myeloid and lymphoid cells involved in host defense and immune response (*9,10*). On the other hand, chemical-induced lethality to both stromal macrophages and fibroblasts could result in such an absence of cytokines and growth factors in the bone marrow that committed and immature hemopoietic progenitors would die from apoptosis (*11*), resulting in an aplastic state. Recent studies with radiation-induced bone marrow toxicity have emphasized the significance of alterations of the stromal microenvironment in the development of aplastic anemia (*12*). Similarly, an altered stromal microenvironment has been linked with the development of leukemia (*11*).

The difference in response of stromal macrophage and fibroblasts to toxicants could be a reflection of the concentration of the chemical (*13*) or the mechanism of toxicity involved (*14*). In this regard, stromal macrophages are more susceptible to the toxic effects of low concentrations of HQ than are stromal fibroblasts (*10*). However, as the concentration of HQ is increased, stromal fibroblasts also succumb to the cytotoxic effects. The basis for this difference in susceptibility to HQ between stromal macrophages and fibroblasts can be attributed in part to macrophages having lower QR activity than stromal fibroblasts (*13,15*) and to macrophages exhibiting a peroxide dependent mechanism capable of activating HQ to a covalent binding species, presumably benzoquinone (*15*). HQ can be oxidized to benzoquinone by myeloperoxidase (*16*), prostaglandin synthase (*17*), superoxide dismutase (*18*) and copper (*19*). Reflective of the cellular conversion of HQ to benzoquinone is the depletion of cellular GSH, which precedes cell toxicity and death (*20*). Since cellular GSH is a factor that modulates the molecular interactions of benzoquinone (Figure 2), it is a determinant of susceptibility to HQ. As illustrated in Figure 4, differences in stromal cell QR and GSH are reflected in the susceptibility of stromal cells from mice and rats to HQ (*21*). *In vivo* studies have shown DBA/2 mice to be more sensitive to benzene-induced hematotoxicity than C57BL/6 mice (*22*) and mice in general are considered more sensitive than rats (*23*). In this regard, the pattern of benzene metabolism in rats is different from that in mice (*24*). Rats produce a fewer number as well as a smaller amount of metabolites that contribute to toxic reactions. The data presented in Figure 4 also indicate that the bone marrow stromal cells of rats are inherently more resistant to both HQ and benzoquinone than those from mice (*21*).

The overall function of the bone marrow is to provide blood and immune cells to the body. This function is accomplished through the differentiation of committed progenitor and precursor cells.

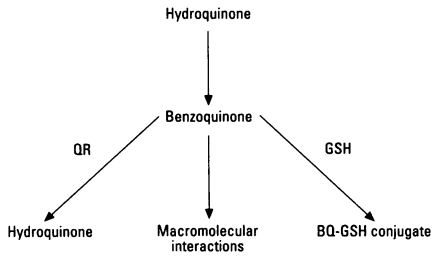

Figure 2. The role of quinone reductase and glutathione in modulating the molecular interactions of benzoquinone resulting from the oxidation of hydroquinone.

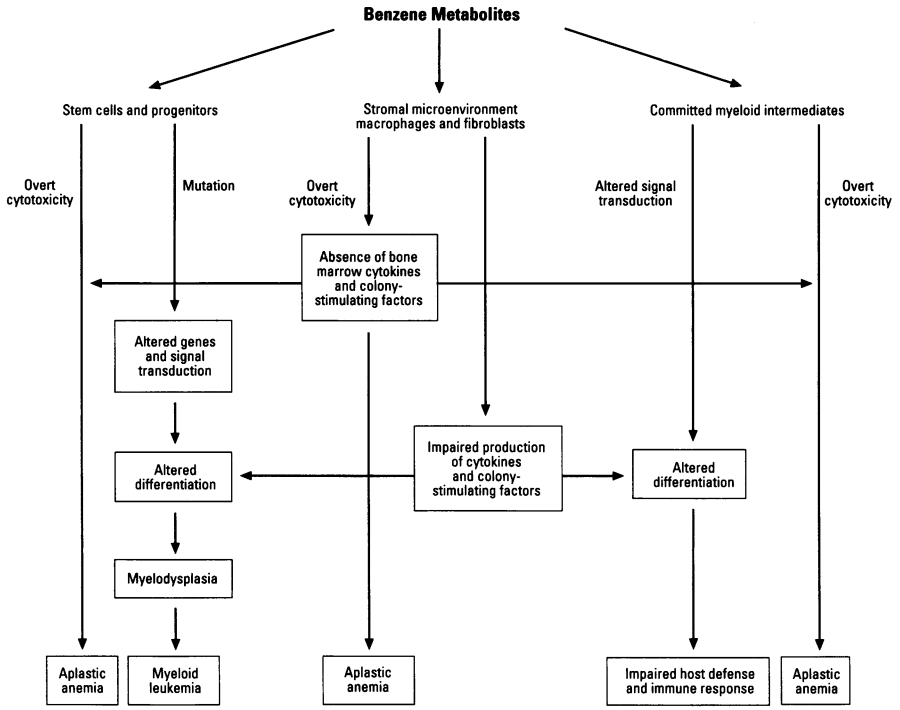

Figure 3. Ways in which modulation of various bone marrow populations by benzene metabolites could result in the induction of aplastic anemia, leukemia, and immunotoxicity.

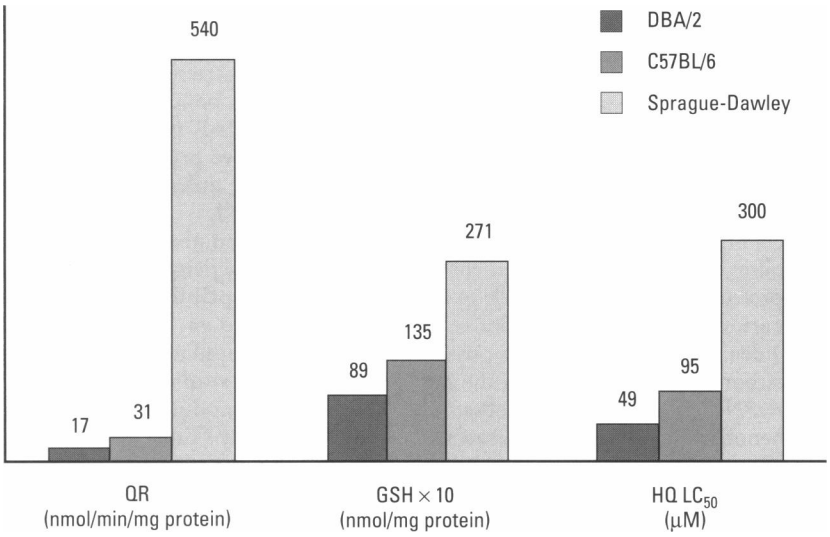

Figure 4. Comparison of the quinone reductase activity, the glutathione content, and the hydroquinone concentration cytotoxic to 50% (LC_{50}) of bone marrow stromal cells from DBA/2 and C57BL/6 mice and Sprague-Dawley rats. Data are summarized from data presented in Twerdok et al. (*13*) and Zhu et al. (*21*).

Our understanding of the biochemical and molecular processes involved in the differentiation of myeloid cells has been advanced through the utilization human leukemic cell lines such as HL-60 and ML-1 cells (*25*). *In vitro*, these two myeloid cell lines continuously proliferate in suspension culture; however, upon addition of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) or 1,25-dihydroxyvitamin D_3, they differentiate into monocytes/macrophages. Conversely, DMSO or retinoic acid induce their differentiation into polymorphonuclear cells. Recently, these myeloid cell lines have been utilized in toxicological studies (*26–28*). The data presented in Table 1 show that HL-60 cells have more GSH than ML-1 cells. On the other hand, the HL-60 cells are relatively QR deficient. When challenged *in vitro* with HQ, the HL-60 cells are more sensitive to the cytotoxic effects of HQ than are the ML-1 cells. GSH depletion occurs at a significantly faster rate in the ML-1 cells than in the HL-60 cells, although HL-60 cells have higher myeloperoxidase activity than ML-1 cells (*29*). On the other hand, ML-1 cells are larger and have more protein that could serve as nonspecific targets for benzoquinone.

We have also compared the response to HQ of undifferentiated and TPA-differentiated ML-1 cells (*30*). In this model, 0.3 ng/ml of TPA is added to 3×10^5 cells/ml seeded in fetal bovine serum (FBS)-coated flasks (Sigma, St. Louis, MO) and cultured in RPMI 1640 media (GIBCO, Grand Island, NY) containing 7.5% FBS. After 3 days, the TPA is washed out, fresh media added, and differentiation allowed to continue for an additional 3 days. As presented in Table 2, the TPA-differentiated cells have significantly more GSH and QR activity. Concomitantly, the TPA-differentiated cells are less susceptible to the cytotoxic effects of HQ than the undifferentiated cells.

The above results with bone marrow stromal cells and the HL-60 and ML-1 cells are consistent with the concept that GSH and QR are biochemical determinants of cellular toxicity to HQ. Accordingly, we have chemically modulated QR and GSH through the actions of dicumoral and buthionine sulfoximine (BSO), respectively (*13,20,21,29,30*). As shown in Table 3, in stromal cells derived from both DBA/2 mice and Sprague-Dawley rats, the inhibition of QR by dicumoral enhanced the toxic effects of HQ. Similarly, dicumoral enhanced the toxicity of HQ to TPA-differentiated ML-1 cells but not the undifferentiated cells, although QR activity was inhibited by dicumoral in the undifferentiated cells (*29*). Thus, in undifferentiated myeloid cell intermediates, and possibly even in stem cell and progenitor cell populations, GSH may be the major defense against benzoquinone, although QR enzyme activity is measurable. QR can utilize either NADPH or NADH as co-factors; however, the activity of QR may be limited by the availability of such co-factors due to the inability of undifferentiated cells to synthesize sufficient quantities of these pyridine dinucleotides. One of the major biochemical differences between undifferentiated and TPA-differentiated ML-1 cells is the relative absence of mitochondrial respiration in the undifferentiated cells (*31*). Further studies are needed to better understand the biochemistry of these undifferentiated cells with regard to matters relevant to mechanisms of chemical-induced toxicity.

The inhibition of GSH synthesis by BSO results in the cellular depletion of GSH and a potentiation of HQ-induced toxicity to DBA/2-derived stromal cells (*20*) and undifferentiated HL-60 and ML-1 cells (*29*). Although GSH content was significantly decreased by BSO in Sprague-Dawley-derived stromal cells and TPA-differentiated ML-1 cells, there was no potentiation of HQ-induced toxicity in these cells (*21,30*). Treatment with BSO resulted in a significant induction in QR activity in the rat stromal cells and the TPA-differentiated ML-1 cells. Shertzer et al. (*32*) recently reported a similar effect of BSO in mouse Hepa-1c1c7 cells. Overall, the data presented in Table 3 are consistent with the concept that QR and

Table 1. Comparison of cellular GSH content, QR activity, and HQ LC_{50} in HL-60 or ML-1 cells.

Parameter	Cell type	
	HL-60	ML-1
GSH, nmol/mg protein	31.7	24.8
QR, nmol/min/mg protein	0.6	16.2
HQ LC_{50}, μM	30.0	45.0

Data summarized from Li et al. (*29*).

Table 2. Comparison of cellular GSH content, QR activity, and HQ LC_{50} between undifferentiated and differentiated ML-1 cells.

Parameter	Cell type	
	Undifferentiated	Differentiated
GSH, nmol/10^6 cells	2.0	24.0
QR, nmol/min/10^6 cells	4.6	57.3
HQ LC_{50}, μM	40.0	200.0

Table 3. Effects of dicumarol and BSO on hydroquinone-induced toxicity.

Cell type	Dicumarol	BSO
DBA/2-derived stromal cells	↑	↑
Sprague-Dawley-derived stromal cells	↑	↔
HL-60 cells	—	↑
ML-1 cells	↔	↑
ML-1 differentiated cells	↑	↔

↑ indicates that the treatment resulted in an increase in HQ-induced toxicity and ↔ indicates that the treatment did not result in a significant change in HQ-induced toxicity. Data summarized from Zhu et al. (*21*), Li and Trush (*20*), and Li et al. (*29*).

GSH are involved in protecting bone marrow cells from the toxic effects of the benzene metabolite HQ.

Chemoprotection against Chemical-induced Disease

Overview

Over the last decade, a greater emphasis has been put on preventing human disease development. This has resulted in the development of molecular interventions that either prevent the initial induction of disease or intervene in the progression of disease. In environmental health, this concept has its origins in studies on the chemoprotection against cancer. Chemoprotection is defined as the protection from the toxicity of one chemical by the administration of another chemical. This phenomenon can be traced to observations made by Berenblum about the skin more than 60 years ago (*33*). Since that time, a plethora of structurally unrelated compounds, including phenolic antioxidants, coumarins, azo dyes, flavonoids, dithiothiones, and isothiocyanates have been demonstrated to protect laboratory animals against the carcinogenic or toxic effects of environmental chemicals (*34,35*).

Mechanistically, the chemoprotective actions of many of the above classes of chemicals lie in their ability to induce the synthesis of enzymes involved in phase II reactions as well as glutathione (*36*). Accordingly, induction of these enzymes alters conditions responsible for target organ toxicity by shifting xenobiotic metabolism, either in the liver or in the target organ, away from the generation of reactive intermediates or toward detoxification. In this regard, Talalay and co-workers have classified chemicals as being bifunctional or monofunctional inducers (*37*), with monofunctional inducers being those that elevate primarily phase II enzymes as well as glutathione and quinone reductase without significantly inducing phase I enzymes such as the cytochrome P450s. Many of the monofunctional inducers also elicit the induction of protective systems without interacting with the Ah receptor, which is a characteristic not shared by many of the bifunctional inducers. The application of chemoprotective strategies to targeted human populations has been further realized as a result of the development of molecular biomarkers and the incorporation of these biomarkers into epidemiologic studies (*38*). As mentioned previously, an excellent example of the development of a mechanistically based chemoprotective strategy is the studies in animal models that utilize oltipraz, a dithiothione, as the chemoprotective agent and several biomarkers for aflatoxin (*39*).

Chemoprotection of the Bone Marrow

Although the liver, lung, and skin are often the target organs examined in chemoprotective studies, several studies suggest that a chemoprotective strategy is applicable to the bone marrow. For example, Huggins et al. (*40,41*) demonstrated that azo dyes prevent hydrocarbon-induced leukemia in the rat. Similarly, Nebert and co-workers observed that phenobarbital given orally protected against the bone marrow toxicity and lethality elicited by dietary benzo[*a*]pyrene (B[*a*]P) in DBA/2 mice with Ah^d/Ah^d genotypes (*42*). In mice with this genotype, administration of B[*a*]P in the diet elicits severe bone marrow depression resembling aplastic anemia (*42,43*). Chronic administration of a low concentration of B[*a*]P results in leukemia (*44*). Phenobarbital administration also reduced the covalent binding of B[*a*]P equivalents in the bone marrow. The basis for the protective effects of phenobarbital against oral B[*a*]P-induced bone marrow toxicity has been attributed to its ability to induce phase II enzymes, thus altering the toxicokinetics of B[*a*]P. Rao et al. (*45*) have also observed that vitamin A given to Swiss mice orally decreased B[*a*]P-induced chromosomal damage in bone marrow. Similarly, a polyphenol containing tannin mixture extracted from green tea suppressed chromosome aberrations induced by aflatoxin B_1 in rat bone marrow cells (*46*). Currently there is much interest in the chemoprotective properties of green tea and its possible utilization by humans for this purpose (*47*).

As described above, it appears that GSH and QR activity are cellular determinants of susceptibility of HQ-induced cytotoxicity to various bone marrow-derived cell populations. This was supported by the studies utilizing BSO or dicumarol to modulate cellular GSH or QR, respectively (Table 3). Therefore, we reasoned that the induction of QR and/or GSH in target bone marrow-derived cells may result in a chemoprotective effect against HQ-induced toxicities. Based on studies by Talalay and co-workers (*35*), with *tert*-butylhydroquinone (*t*-BHQ) we demonstrated that the induction of QR resulted in protection against HQ-induced cytotoxicity to stromal cells obtained from C57BL/6 or DBA/2 mice (*48*). Although *t*-BHQ is an effective inducer, it also has cytotoxic effects that limit its usefulness for this purpose. Thus, 1,2-dithiole-3-thione (D3T) was used as the monofunctional inducer in subsequent studies (*13,21,29,49*). The data summarized in Figure 5, demonstrate that D3T is an effective inducer of QR and GSH in mouse and rat bone marrow stromal cells and the HL-60 and ML-1 cell lines. Accordingly, in these cells there

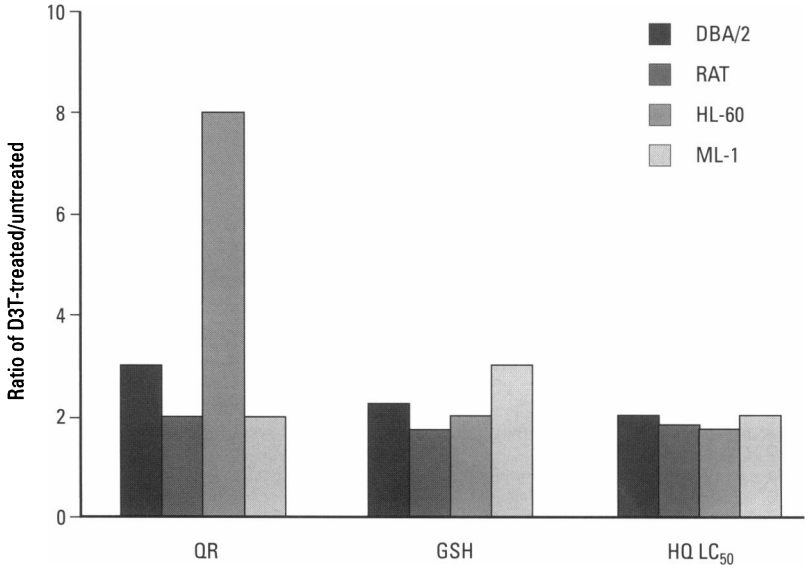

Figure 5. Effects of 1,2-dithole-3-thione on the quinone reductase, the glutathione content, and the LC_{50} of hydroquinone of stromal cells from DBA/2 mice and Sprague-Dawley rats and the HL-60 and ML-1 myeloid cell lines. Data summarized from data presented by Zhu et al. (*21*) and Li et al. (*29*).

is a protection against the cytotoxic effects of HQ. While constitutive expression of QR is relatively deficient in HL-60 cells, this enzyme can be induced 8-fold by D3T treatment. However, the induced QR activity of HL-60 cells is still not equivalent to the basal activity of ML-1 cells (*29*). In addition, while D3T is an effective inducer of glutathione *S*-transferase (GST) activity in mouse or rat liver (*37,50*), GST is not induced to a significant extent in either mouse or rat bone marrow-derived stomal cells (*13,21*) or the HL-60 or in ML-1 cells (*29*).

Previous studies have noted that HQ is selectively toxic to mouse bone marrow stromal macrophages (*9,10*). At noncytotoxic concentrations, HQ inhibits the synthesis of IL-1, thereby interfering with the ability of stromal macrophages to communicate with stromal fibroblasts, the cells which are responsible for releasing granulocyte macrophage colony stimulating and IL-3. Thus, as a result of inhibiting the synthesis of IL-1, HQ can inhibit both myelopoiesis and lymphopoiesis (*9,10*). Renz and Kalf (*51*) have shown that the administration of recombinant IL-1 overcomes the bone marrow suppressive effects of benzene. We have also observed that *in vitro* D3T treatment can prevent the effects of HQ in suppressing the ability of stromal cells to support colony-forming activity (*49*). Treatment of DBA/2 mouse-derived stromal cells with a noncytotoxic concentration of HQ inhibits colony-forming activity by nearly 50% (Table 4). It has previously been demonstrated that stromal fibroblasts can maintain hematopoiesis 50% in the absence of functional macrophages (*9,10*). Moreover, as shown in Table 4, stromal cells treated with HQ maintain their full function when pretreated with D3T.

Table 4. D3T protection against HQ-induced inhibition of DBA/2 stromal cell-conditioned medium to support CFU–G/M colony formation.

Source of CSA	Stromal cell treatment	No. colonies
None	NA	2
Stromal medium	None	361
Stromal medium	15 μM HQ	204
Stromal medium	75 μM D3T pretreatment followed by 15 μM HQ	367

Abbreviations: CSA, colony-stimulating activity; NA, not applicable. Conditioned medium was obtained from stromal cells from DBA/2 mice cultured for 12 days in the presence of HQ or HQ/D3T. After the conditioned medium was concentrated by centrifugation in centricells, the CSA of the media was determined as described in Twerdok et al. (*49*).

D3T can also protect against the inhibition by HQ of the differentiation of ML-1 cells to monocytes and macrophages (*52*). In these studies we used mitochondrial maturation as a marker of the differentiation of monocytes/macrophages (*31,53*) by monitoring mitochondrial-dependent lucigenin-derived chemiluminescence (*54,55*). Shown in Figure 5, treatment with D3T induces QR and GSH in undifferentiated ML-1 cells. As such, in D3T-treated cells, the inhibition of cell division and viability by HQ is reduced (*29*). Likewise, treatment of ML-1 cells with 10 μM HQ for 3 hr prior to the induction of differentiation by TPA results in altered differentiation (*52*) similar to that seen with HL-60 cells (*26*). Pretreatment of the ML-1 cells with BSO increases their susceptibility to the differentiation-altering effects of HQ (Figure 6). Conversely pretreatment with D3T, which induces GSH, prevents the differentiation-altering effects of HQ. These observations suggest that the interaction of benzoquinone with a sulfhydryl-containing target molecule involved in signal transduction may underlie the effects of HQ on TPA-induced differentiation of ML-1 cells to monocytes/macrophages.

The above *in vitro* studies with bone marrow-derived stromal cells and the ML-1 cells further demonstrate that QR and GSH are determinants of susceptibility to HQ and indicate that induction of QR and GSH by D3T may be a useful chemoprotective strategy against benzene-induced hematoxicities. As such, *in vivo* D3T feeding to DBA/2 mice was undertaken to determine if D3T has an inductive effect in the bone marrow (*49*). The data presented in Table 5 indicate that feeding 0.1% D3T in the diet for 6 days resulted in a significant induction of QR activity in bone marrow stromal cells. Moreover, when challenged *ex vivo* with 50 μM HQ, the cells from the D3T-fed mice were less susceptible to the cytotoxic effects of HQ than cells from control mice. These results indicate that it is possible through feeding D3T to induce protective systems within the bone marrow compartment. Although only the cytotoxic effect of HQ was examined in these cells from D3T-fed mice, one would expect that they would also be less susceptible to the inhibitory effects of HQ on cytokine synthesis, which occurs at a lower concentration (Table 4). Clearly, further studies are warranted to determine if D3T can protect against the *in vivo* bone marrow effects of benzene or benzene-derived metabolites. It should be appreciated that a chemoprotective effect of D3T *in vivo* may reflect the inductive effects of D3T not only in the bone marrow, the target organ, but also in

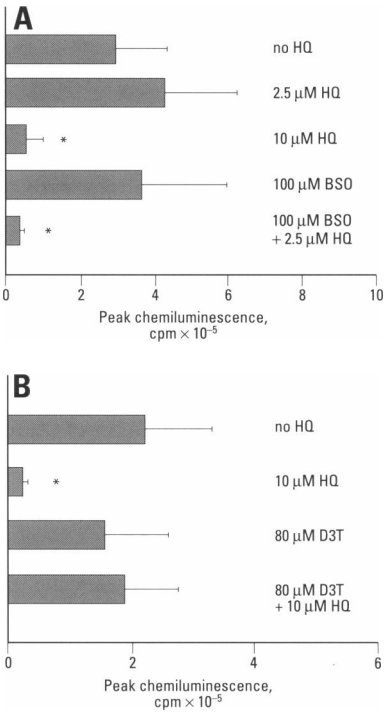

Figure 6. Effects of glutathione modulation on the effect of hydroquinone (HQ) on the TPA-induced differentiation of ML-1 cells. In these experiments differentiation was assessed by mitochondrial maturation as monitored by peak mitochondrial-dependent lucigenin-derived chemiluminescence (*31,54,55*). (*A*) Cells were pretreated with 100 μM BSO for 24 hr prior to initiation of differentiation by TPA (0.3 ng/ml). (*B*) Cells were pretreated with 80 μM D3T for 24 hr prior to initiation of differentiation by TPA. In these experiments, undifferentiated cells were treated with HQ for 3 hr, the HQ removed, and the TPA added. The results are mean ± SD of three to four experiments. Asterisk (*) indicates significantly different ($p < 0.05$) from no HQ as determined by Student's *t*-test.

Table 5. Effects on stromal cell quinone reductase activity and hydroquinone toxicity of feeding D3T to DBA/2 mice.

	Diet	
	Control	0.1% D3T
QR activity, nmole/min/mg protein	30	46
Cell survival, %, following *ex vivo* addition of 50 μM HQ	37	62

DBA/2 mice were fed 0.1% D3T in the diet for 6 days. Subsequently, bone marrow was removed, the stromal cells were allowed to adhere for 24 hr and QR activity determined. Flushed cells were also treated with 50 μM QR for 24 hr, and the survival of the attached (live) stromal cells determined as described in Twerdok et al. (*49*).

the liver, where the metabolism of benzene occurs. Of particular significance in this regard is the role of glutathione *S*-transferase, QR, and glucuronosyltransferase in benzene metabolism. Thus, the effect of D3T in the bone marrow could be viewed as modulating the toxicodynamics of benzene-derived metabolites, while its effect in the liver would tend to modulate the toxicokinetics of benzene.

Identifying Individuals Susceptible to Benzene for Chemoprotective Studies

As with all potentially hazardous chemicals, the best way to protect humans from the chronic low-dose toxic effects of benzene is to sufficiently reduce or, if possible, eliminate exposure. Presently, cigarette smoke is the major source of benzene exposure for the general population (*2*). Even under the best of exposure scenarios, there still may be individuals who are so inherently susceptible to toxic effects of benzene that some mechanistically based chemoprotective strategy may be warranted. The issue then becomes how to identify these susceptible individuals within populations. It is clear from a number of studies that benzene-induced hematotoxicity is determined by the metabolism and processing of benzene in both the liver and the bone marrow (*5,7,13,15*). In the liver the major susceptibility determinant is CYP2E1, since it is the isoform of cytochrome P450 that catalyzes the formation of phenol and HQ (*6,7*). It is the further processing of these metabolites to quinone derivatives and muconaldehyde that has been most implicated in benzene-induced toxicity (*5*). Studies have shown that there are significant differences in the CYP2E1 activity of human microsomes that are reflected in their metabolism of benzene (*7,56*). Moreover, CYP2E1 is inducible by alcohol exposure, which has been shown to increase the risk for benzene-induced toxicity (*6*). Thus, it is reasonable to expect that within the human population there are individuals who have high CYP2E1 levels and others who have low CYP2E1 levels. Individuals with high CYP2E1 activity would have the ability to produce more hydroxylated benzene derivatives and as such could be viewed as being more susceptible. However, the bioactivities of these metabolites could be balanced by the protective and detoxifying systems present in the various bone marrow cell populations.

Among the liver-derived metabolites of benzene, HQ has been shown to accumulate in the bone marrow of mice, a susceptible species (*24*). Within bone marrow cells are a number of possible mechanisms that could convert HQ to the bioreactive benzoquinone (*16–19*). As pointed out (above), once benzoquinone is formed within cells, its ability to interact with target molecules is determined by two factors, QR activity and GSH (Figure 2). The cellular activity of QR and availability of GSH could also be relevant to the bioreactivity of catechol and benzenetriol, other polyhydroxylated metabolites of benzene, while GSH would also be relevant to the biological actions of muconaldehyde. As was presented in Figure 4 and Table 1, there are significant differences in QR and GSH between different strains of mice, between mice and rats, and between two model human myeloid cell lines. In addition, there appear to be differences in QR and GSH between undifferentiated and differentiated ML-1 cells (Table 2). Therefore, it is also reasonable to expect that there could be significant variations between humans with regard to bone marrow QR and GSH that could contribute to their relative susceptibility to benzene.

Both hepatic CYP2E1 and bone marrow QR and GSH should be taken into consideration in determining the relative susceptibility of individuals to benzene (Figure 7). As such, individuals with high hepatic CYP2E1 and low bone marrow QR and GSH could be viewed as being the most susceptible within a population. Conversely, those individuals with low hepatic CYP2E1 and high bone marrow QR and GSH could be viewed as being the least susceptible within the population. As research continues on understanding the mechanisms involved in benzene-induced hematotoxicity (Figures 1,3), additional factors in the bone marrow could be considered pertinent to susceptibility. Nonetheless, presently it is possible to phenotype individuals for CYP2E1 activity through chlorzoxazone hydroxylation (*57,58*). For the overall assessment of bone marrow QR and GSH, cells obtained from a bone marrow biopsy would be the most relevant. Considering the invasiveness of this procedure, this would not be practical. However, it is quite possible that peripheral human monocytes and polymorphonuclear leukocytes could be used as surrogates for bone marrow stromal macrophages and committed myeloid intermediates, respectively, to obtain a relevant assessment of QR and GSH studies. There is an increase in QR and GSH with mononuclear differentiation (Table 2). On the other hand, we have observed that the DMSO-induced differentiation of HL-60 cells along the polymorphonuclear pathway is not accompanied by similar increases in QR and GSH (Y Li and MA Trush, unpublished observations). Thus, it should be possible to identify individuals who may differ in their susceptibility to benzene; depending upon their exposure to benzene, those considered more susceptible could be candidates for some mechanistically based molecular intervention. As the data in this article have pointed out, D3T is able to induce QR and GSH in several bone marrow-derived cell populations and *in vitro* significantly modify several benzene metabolite-induced toxicities. As such, given the development of oltipraz, a dithiothione, as a potential human chemoprotective agent in a targeted population (*3*), a similar approach could be used in benzene-exposed populations.

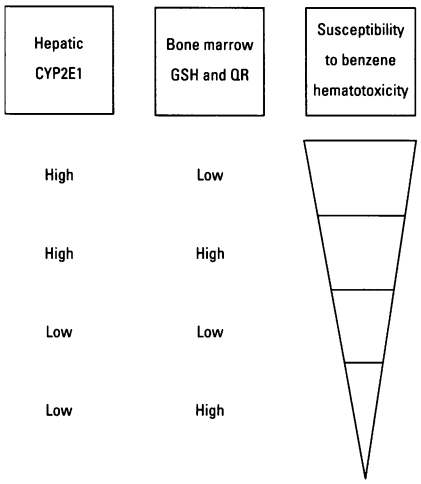

Figure 7. Ways in which susceptibility to benzene-induced hematotoxicity may be related to variations in hepatic CYP2E1 activity and bone marrow QR and GSH.

REFERENCES

1. Hunter D. The Diseases of Occupations. 6th ed. London:Hodder and Stoughton, 1978.
2. Paustenbach DJ, Bass DR, Price P. Benzene toxicity and assessment, 1991–1992: implications for future regulation. Environ Health Perspect Suppl 101:177–200 (1993).
3. Kensler TW, Helzlsouer KJ. Oltripraz: clinical opportunities for cancer chemoprevention. J Cell Biochem, Suppl 22:101–107 (1995).
4. Greenburg L. Results of medical examination and clinical tests made to

discover early signs of benzol poisoning in exposed workers. Public Health Rep 41:1526–1539 (1926).
5. Snyder R, Longacre SL, Witmer C, Kocsis JJ, Andrews LS, Lee EW. Biochemical toxicology of benzene. In: Reviews in Biochemical Toxicology, Vol 3 (Hodgson E, Bend JR, Philpot RM, eds.) New York:Elsevier, 1981;123–153.
6. Johansson I, Ingelman-Sundberg M. Benzene metabolism by ethanol-, acetone-, and benzene-inducible cytochrome P-450 (IIE1) in rat and rabbit liver microsomes. Cancer Res 48:5387–5390 (1988).
7. Seaton MJ, Schlosser PM, Bond JA, Medinsky MA. Benzene metabolism by human liver microsomes in relation to cytochrome P-450 2E1 activity. Carcinogenesis 15:1799–1806 (1994).
8. Williams WJ, Bentler E, Erslev AJ, Lichtiman MA. Hematology. 3rd ed. New York:McGraw-Hill, 1983.
9. King AG, Landreth KS, Wierda D. Hydroquinone inhibits bone marrow pre-B cell maturation *in vitro*. Mol Pharmacol 32:807–812 (1987).
10. Thomas DJ, Reasor MJ, Wierda D. Macrophage regulation of myelopoiesis is altered by exposure to the benzene metabolite hydroquinone. Toxicol Appl Pharmacol 97:440–453 (1989).
11. Long MW, Wicha MS, eds. The Hematopoietic Microenvironment: the Functional and Structural Basis of Blood Cell Development. Baltimore:Johns Hopkins University Press, 1993.
12. Jones TD, Morris MD, Young RW, Kehlet RA. A cell kinetics model for radiation-induced myelopoiesis. Exp Hematol 21:816–822 (1993).
13. Twerdok LE, Rembish SJ, Trush MA. Induction of quinone reductase and glutathione in bone marrow cells by 1,2-dithiole-3-thione: effect on hydroquinone-induced cytotoxicity. Toxicol Appl Pharmacol 12:273–281 (1992).
14. Zhu H, Li Y, Trush MA. Characterization of benzo[*a*]pyrene quinone-induced toxicity to primary cultured bone marrow stromal cells from DBA/2 mice: potential role of mitochondrial dysfunction. Toxicol Appl Pharmacol 13:108–120 (1995).
15. Thomas DJ, Sadler A, Subrahmanyam VV, Siegel D, Reasor MJ, Wierda D, Ross D. Bone marrow stromal cell bioactivation and detoxification of the benzene metabolite hydroquinone: comparison of macrophages and fibroblastoid-cells. Mol Pharmacol 37:255–262 (1990).
16. Subrahmanyam VV, Kolachana P, Smith MT. Metabolism of hydroquinone by myeloperoxidase: mechanisms of stimulation by other phenolic compounds. Arch Biochem Biophys 156:759–763 (1991).
17. Schlosser MJ, Surina RD, Kalf GF. Metabolism of phenol and hydroquinone to reactive products by macrophage peroxidase or purified prostaglandin H-synthase. Environ Health Perspect 82:229–237 (1989).
18. Li Y, Trush MA. Oxidative stress and its relationship to carcinogen activation. In: Oxidative Stress and Aging (Culter R, Packer L, Bertram J, Mori A, eds). Basel:Birkhuser Verlag, 1995;203–220.
19. Li Y, Trush MA. Oxidation of hydroquinone by copper: chemical mechanism and biological effects. Arch Biochem Biophys 300:346–355 (1993).
20. Li Y, Trush MA. Alteration of cellular glutathione as a factor in hydroquinone-induced cytotoxicity to primary cultured bone marrow stromal cells from DBA/2 mice. In Vitro Toxicol 5:59–71 (1992).
21. Zhu H, Li Y, Trush MA. Differences in xenobiotic detoxifying activities between bone marrow stromal cells from mice and rats: implications for benzene-induced hematotoxicity. J Toxicol Environ Health, 46:183–201 (1995).
22. Longacre SL, Kocsis JJ, Snyder R. Influence of strain differences in mice on the metabolism and toxicity of benzene. Toxicol Appl Pharmacol 60:398–409 (1981).
23. NTP. National Toxicology Program Technical Report on the Toxicology and Carcinogenesis Studies of Benzene (CAS No. 71-43-2) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Research Triangle Park, NC:National Toxicology Program, 1986.
24. Sabourin PJ, Bechtold WE, Birnbaum LS, Lucier G, Henderson RF. Differences in the metabolism and disposition of inhaled [^{3}H]benzene by F344/N rats and B6C3F$_1$ mice. Toxicol Appl Pharmacol 94:128–140 (1988).
25. Lyons AB, Ashman LK. Monocyte cell lines. In: Human Monocytes (Zembola M, Asherson GL, eds). San Diego: Academic Press, 1989;59–69.
26. Oliveria NL, Kalf GF. Induced differentiation of HL-60 promyelocytic leukemia cells to monocyte/macrophages by hydroquinone, a hematotoxic metabolite of benzene. Blood 79:627–633 (1992).
27. Levay G, Pongracz K, Modell WJ. Detection of DNA adducts in HL-60 cells treated with hydroquinone and *p*-benzoquinone by ^{32}P-postlabeling. Carcinogenesis 12:1181–1186 (1991).
28. Chuang LF, Hinton DE, Cheung ATW, Chuang RY. Induction of differentiation in human myeloblastic leukemia ML-1 cells by heptachlor, a chlorinated hydrocarbon insecticide. Toxicol Appl Pharmacol 109:98–107 (1991).
29. Li Y, Lafuente A, Trush MA. Characterization of quinone reductase, glutathione and glutathione *S*-transferase in human myeloid cell lines: induction by 1,2-dithiole-3-thione and effects on hydroquinone-induced cytotoxicity. Life Sci 54:901–916 (1994).
30. Li Y, Zhu H, Trush MA. 12-*O*-Tetradeconylphorbol-13-acetate (TPA)-induced differentiation to monocyte/macrophages is accompanied by increased xenobiotic detoxifying activities and decreased susceptibility to hydroquinone (HQ)-induced toxicity. Toxicologist 15:302 (1995).
31. Rembish SJ, Craig RW, Trush MA. Characterization of an *in vitro* model for assessing mitochondrial maturation in monocytic cells. Toxicologist 12:284 (1992).
32. Shertzer HG, Vasiliou V, Liu R-M, Tabor MW, Nebert DW. Enzyme induction by L-buthionine (SR)-sulfoximine in cultured mouse hepatoma cells. Chem Res Toxicol 8:431–436 (1995).
33. Berenblum I. The modifying influence of dichlorethyl sulphide on the induction of tumors in mice by tar. J Pathol Bacteriol 32:425–434 (1929).
34. Wattenberg LW. Chemoprevention of cancer. Cancer Res 45:18 (1985).
35. DeLong MJ, Prochaska HJ, Talalay P. Induction of NAD(P)H:quinone reductase in murine hepatoma cells by phenolic antioxidants, azo dyes, and other chemoprotectors: a model system for the study of anticarcinogens. Proc Natl Acad Sci USA 83:787–791 (1986).
36. Talalay P, DeLong MJ, Prochaska HJ. Molecular mechanisms in protection against carcinogenesis. In: Cancer Biology and Therapeutics (Cory JG, Szentivani A, eds). New York:Plenum Press, 1987;197–216.
37. Prochaska HJ, Talalay P. Regulation mechanism of monofunctional and bifunctional anticarcinogenic enzyme inducers in murine liver. Cancer Res 48:4776–4782 (1988).
38. Qian G-S, Ross RK, Yu MC, Yuan J-M, Gao Y-T, Henderson BE, Wogan GN, Groopman JD. A follow-up study of urinary markers of aflatoxin exposure and liver-cancer risk in Shanghai, People's Republic of China. Cancer Epidemiol Biomarkers Prev 3:3–10 (1994).
39. Groopman JD, DeMatos P, Egner PA, Love-Hunt A, Kensler TW. Molecular dosimetry of urinary aflatoxin-N^7-quanine and serum-albumin adducts predicts chemoprotection by 1,2-dithiole-3-thione in rats. Carcinogenesis 13:101–106 (1992).
40. Huggins CB, Ueda N, Russo A. Azo dyes prevent hydrocarbon-induced leukemia in the rat. Proc Natl Acad Sci USA 75:4524–4527 (1978).
41. Huggins C, ed. Experimental Leukemia and Mammary Cancer. Induction, Prevention and Cure. Chicago:University of Chicago Press, 1979.
42. Nebert DW, Jensen NM, Levitt RC, Felton JS. Toxic chemical depression of the bone marrow and possible aplastic anemia explainable on a genetic basis. Clin Toxicol 16:99–122 (1980).

43. Nebert DW, Levitt RC, Jensen NM, Lambert GH, Felton JS. Birth defects and aplastic anemia: differences in polycyclic hydrocarbon toxicity-associated with the *Ah* locus. Arch Toxicol 39:109–132 (1977).
44. Nebert DW, Jensen NM. Benzo[*a*]pyrene-initiated leukemia in mice associated with allelic differences at the *Ah* locus. Biochem Pharmacol 27:149–151(1970).
45. Rao KP, Nandan BD. Modification of benzo[*a*]pyrene induced chromosomal damage in mouse bone marrow by vitamin A. Bull Environ Contam Toxicol 45:829–832(1990).
46. Ito Y, Ohnishi S, Fujie K. Chromosome aberrations induced by aflatoxin B1 in rat bone marrow cells *in vivo* and their suppression by green tea. Mutation Res 222:253–261 (1989).
47. Yang CS, Wang Z-Y. Tea and cancer. J Natl Cancer Inst 85:1038–1049,(1993).
48. Twerdok LE, Trush MA. Differences in quinone reductase activity in primary bone marrow stromal cells derived from C57BL/6 and DBA/2 mice. Res Commun Chem Pathol Pharmacol 67:375–386 (1990).
49. Twerdok LE, Rembish SJ, Trush MA. Studies with 1,2-dithiole-3-thione as a chemoprotector of hydroquinone-induced toxicity to DBA/2-derived bone-marrow stromal cells. Environ Health Perspect 101:172–177 (1993).
50. Kensler TW, Groopman J, Roebuck BD. Chemoprotection by oltripraz and other dithiothiones. In: Cancer Chemoprevention (Wattenberg L, Lipkin M, Boone-W, Kelloff GJ, eds). Boca Raton, FL:CRC Press, 1992;205–226.
51. Renz JF, Kalf GF. Role for interleukin-1 (IL-1) in benzene-induced hematotoxicity. Inhibition of conversion of pre-IL-α to mature cytokine in murine macrophages by hydroquinone and prevention of benzene-induced hematotoxicity by IL-α. Blood 78:938–944 (1991).
52. Rembish SJ, Craig RW, Trush MA. Characterization of the effects of hydroquinone on ML-1 cell differentiation. Toxicologist 13:424 (1993).
53. He H, Trush MA. Time and concentration dependency of phorbol ester treatment on mitochondrial superoxide generation during differentiation of ML-1 cells. Toxicologist 15:281 (1995).
54. Rembish SJ, Yang Y, Esterline RE, Seacat A, Trush MA. Lucigenin-derived chemiluminescence as a monitor of mitochondrial maturation and modulation in mononuclear cells. In: *In Vitro* Toxicology: Mechanisms and New Technology, Vol 8 (Goldberg AM, ed). New York:Mary Ann Liebert 1991;463–469.
55. Rembish SJ, Trush MA. Further evidence that lucigenin-derived chemiluminescence monitors mitochondrial superoxide generation in rat alveolar macrophages. Free Radic Biol Med 17:117–126 (1994).
56. Seaton MJ, Schlosser PM, Bond JA, Medinsky MA. *In vitro* conjugation of benzene metabolites by human liver: potential influence of interindividual variability on benzene toxicity. Carcinogenesis 16:1519–1527 (1995).
57. Peter R, Bocker RG, Beaune PH, Iwasaki M, Guengerich FP, Yang CS. Hydrolylation of chlorzoxazone as a specific probe for human liver cytochrome P-450 IIE1. Chem Res Toxicol 3:566–573 (1990).
58. Guengerich FP, Kim D-H, Iwasaki M. Role of human P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. Chem Res Toxicol 4:168–179 (1991).