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

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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 mechanically 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.

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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 hematotoxicities.

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,
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 the 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.
Our understanding of the biochemical and molecular processes involved in the differentiation of myeloid cells has been advanced through the utilization of 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 D3, 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 $3 \times 10^5$ 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 dicumonal and buthionine sulfoximine (BSO), respectively (13,20,21,29,30). As shown in Table 3, in

![Figure 4](image)

**Figure 4.** Comparison of the quinine reductase activity, the glutathione content, and the hydroquinone concentration cytotoxicity 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. (19) and Zhu et al. (21).

**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, mmol/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 dicumonal and BSO on hydroquinone-induced toxicity.

| Cell type | Dicumonal | 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 of 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 Abd/Abd 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 B1 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...
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, 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 stromal 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.

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

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

| Source of CSA | Stromal 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 centrtri- cells, the CSA of the media was determined as described in Twerdok et al. (49).

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 isozyme 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 benzene-tol, 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 mouse 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 chlorozoxazone hydroxylsation (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 monocuclear 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 oltripraz, a dithionine, as a potential human chemoprotective agent in a targeted population (3), a similar approach could be used in benzene-exposed populations.

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