# Studies with 1,2-Dithiole-3-thione As a Chemoprotector of Hydroquinone-induced Toxicity to DBA/2-derived Bone Marrow Stromal Cells

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Stromal cells from DBA/2 mouse bone marrow have been shown to be susceptible to cytotoxicity induced by several redox-active metabolites of benzene, including hydroquinone (HQ). Treatment with HQ also alters the composition of stromal cell populations by preferentially killing stromal macrophages compared to stromal fibroblasts. This cytotoxicity can be prevented by 1,2-dithiole-3-thione (DTT) as a result of the induction of quinone reductase (QR), a quinone-processing enzyme, and glutathione. The inductive activities of DTT protected stromal cells against HQ-induced cytotoxicity and against HQ-induced impairment of stromal cell ability to support myelopoiesis. *In vivo* feeding of DTT to DBA/2 mice increased QR activity within the bone marrow compartment and protected bone marrow stromal cells isolated from the DTT-fed animals from *ex vivo* HQ challenge. Thus, the inducibility of cellular defense mechanisms and xenobiotic-processing enzymes by chemoprotective agents such as DTT may be a useful strategy for protecting against chemically induced bone marrow toxicities. **Key words:** bone marrow, chemoprotection, DBA/2, hydroquinone, quinone reductase, stromal cells. *Environ Health Perspect* 101:172–177(1993).

Stromal cells of the bone marrow are a mixed population of interactive cells consisting primarily of fibroblasts and macrophages (*1–3*). These cells are a critical target of toxicity because they are an essential component of the bone marrow microenvironment that contributes to the normal regulation of hematopoiesis (*1,4*). The sensitivity of stromal cells has been observed in a series of experiments demonstrating that hydroquinone (HQ), a redox-active metabolite of benzene, is toxic to the stromal element of the bone marrow (*5*). Thomas and co-workers (*6*) reported that long-term cultures of stromal macrophages are more sensitive to HQ-induced cytotoxicity than a stromal fibroblastoid cell line. Maintaining stromal macrophage viability and function is important because the stromal macrophages synthesize and release interleukin-1, which in turn induces stromal fibroblastoid and endothelial cells to produce colony-stimulating activity and interleukins (IL) for myelopoiesis and lymphopoiesis (*7–9*). Furthermore, noncytotoxic concentrations of HQ, which had no effect on DNA or protein synthesis, prevented conversion of the 34-kD pre-IL-α to mature 17-kD cytokine in purified murine bone marrow macrophages (*10*).

Recently it was shown that quinone reductase (QR) activity and glutathione (GSH) concentration within stromal cells are important determinants of susceptibility to HQ-induced cytotoxicity (*6,11,12*). Quinone reductase is a widely distributed cytosolic flavoprotein important in cellular processing of quinones (*13,14*). In particular, QR catalyzes the two-electron reduction of quinones to hydroquinones, which are then amenable to glucuronidation and excretion (*15,16*). Induction of glutathione as well as phase II enzymes, including QR, is a useful mechanism to enhance the detoxification of some chemically reactive intermediates (*14*). Quinone reductase is coordinately induced with other electrophile-processing phase II enzymes, such as the glutathione-*S*-transferases (*14,17,18*). Recent studies by DeLong and co-workers (*19*) showed that the *in vitro* induction of QR can protect hepatoma cells against the toxicity of a number of quinone xenobiotics. We recently demonstrated the chemoprotective nature of the induction of GSH and QR activity against HQ-induced cytotoxicity in bone marrow stromal cells (*11,12,20*). In these previous studies, we used *tert*-butylhydroquinone (*t*BHQ) and 1,2-dithiole-3-thione (DTT) as inducing agents. In the current study, only DTT was used as the inducing agent because it does not exhibit toxic effects against stromal cells *in vitro* at concentrations up to 250 μM. Additionally, DTT is classified as a monofunctional inducer that does not require interaction with the Ah receptor, which is defective in the DBA/2 strain of mouse, to elicit *in vitro* or *in vivo* GSH and/or enzyme induction (*21,22*).

The purposes of the current study were to determine if DTT protects DBA/2-derived stromal elements against the cytotoxic effects of HQ and if DTT also protects stromal cell functional activity by supporting myelopoiesis (Fig. 1). Additionally, *in vivo* induction of QR activity within the bone marrow compartment was studied by feeding DTT to mice.

## Methods

We obtained male DBA/2 mice (25–30 g) from Jackson Laboratories (Bar Harbor, ME) and housed them in an air-conditioned room (70°F) with a light period from 6 AM to 6 PM. Purina laboratory chow and water were available *ad libitum* to all animals except those fed DTT.

Thomas Kensler of Johns Hopkins University kindly provided the DTT [5-(2-pyrazinyl)]-1,2-dithiole-3-thione (RP 33,851)]. We obtained gridded TC35 tissue culture plates from Lux (Nunc, Inc., Naperville, IL). Microtiter plates and all other tissue culture plasticware were obtained from COSTAR (Van Nuys, CA). We measured absorbencies in 96-well microtiter plates with an automated optical scanner equipped with a 610-nm filter (Biotek, Winooski, VT). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), L-asparagin, penicillin/streptomycin, L-glutamine, trypsin, Dulbecco's phosphate-buffered saline (PBS), and agar were obtained from Gibco (Grand Island, NY). We obtained CentriCell concentrating centrifuge kits (cat. Y18674-2 and Y18674-8) from Polysciences, Inc. (Warrington, PA). The antioxidant-free diet used for DTT feeding studies was powdered AIN-76 purified diet plus menadione without ethoxyquin, and was procured from TEKLAD DIETS (TEKLAD, Madison, WI). We obtained all other

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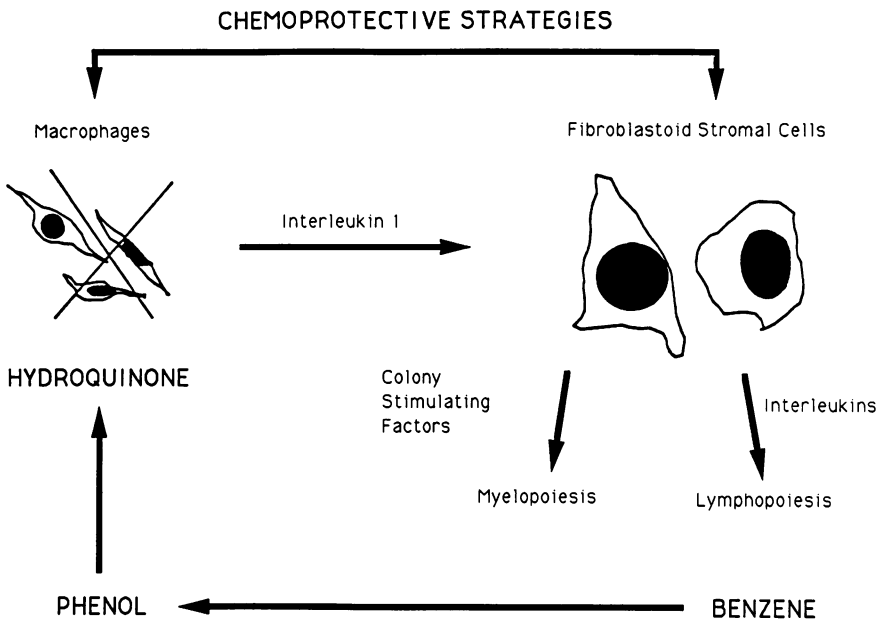


**Figure 1.** Strategies for chemoprotection against benzene and its metabolites in bone marrow, modified from Wierda (*40*).

reagents from the Sigma Chemical Company (St. Louis, MO). We dissolved hydroquinone in PBS immediately before adding it to cell cultures to minimize oxidation.

***Isolation of Bone Marrow Cells and Primary Adherent Stromal Cell Cultures.*** Bone marrow cells were flushed from the femurs of mice according to the method of Oliver and Goldstein (*23*) and pooled in either PBS or DMEM (no additions). The procedure used to establish primary adherent stromal cell cultures was a modification of the method of Zipori and Bol (*24*). For adherent cell culture, we pooled cell suspensions from two or more animals and diluted them with DMEM supplemented with 15% FBS, 2 mM glutamine, 50 μM 2-mercaptoethanol, 100 μg/ml L-asparagin, 100 IU/ml penicillin, and 100 μg/ml streptomycin, and then plated the suspensions on 100-mm tissue culture dishes, six plates per two animals. Twenty-four hours later, we gently aspirated media and replaced it. At 48 hr we washed cultures twice with PBS to remove unattached cells and debris. Cultures were maintained in 100-mm tissue culture dishes, changing media every 5–6 days, until they were used in microtiter plate and other assays 10–18 days after isolation. For all toxicity assays, we trypsinized cells and replated them in 96-well microtiter plates at a concentration of $3 \times 10^4$ cells/well.

***Chemical Protection against Hydroquinone-induced Toxicity in Primary Stromal Cells.*** We plated cells into 96-well plates at $3 \times 10^4$ cells/well and treated them with 75 μM DTT 24 hr later. After another 24 hr, we replaced the chemoprotector with HQ, and 24 hr later (a total of 72 hr after plating), we assayed cells for survival with crystal violet staining as previously described (*11*). Peak induction of QR activity occurs between 16 and 24 hr after a single dose of DTT, and it has been determined that 75 μM DTT induces essentially the maximal QR activities observed in these cells after DTT treatment (*12*). We report survival as percent survival of treated: control groups, with control cells representing 100% [($Abs_{610}$ treated cells/$Abs_{610}$ control cells) ×100]. In studies to determine if DTT could protect against HQ inhibition of stromal cell support of myelopoiesis, we pretreated primary cultures for 24 hr with 75 μM DTT, removed DTT and replaced it with 20 μM HQ, and conditioned and concentrated the medium for CFU-G/M (colony-forming units of granulocytes and monocytes) assays as described below.

***Assessment of Preferential Killing by Hydroquinone of Macrophages versus Fibroblasts in DBA/2-derived Primary Stromal Cells.*** Six replicate TC 60 plates per *n* (*n* refers to one discreet experiment/observation) were seeded with $2 \times 10^6$ 2-week-old stromal cells. One 96-well microtiter plate per *n* was also seeded with $3 \times 10^4$ cells/well. We treated three plates/*n* and three rows of wells/*n* 24 hr later (day 2) with 75 μM DTT. On day 3, we treated one plate or microtiter plate row with 0, 35, or 50 μM hydroquinone (these doses of HQ are the $LC_{25}$ and $LC_{50}$ doses for DBA/2 primary stromal cells). Finally, on day 4, 72 hr after plating, we collected cells in the TC60 plates by scraping and centrifugation and stained them for esterase activity using two different substrates, α-naphthylacetate (nonspecific) and α-naphthylbutyrate (Sigma diagnostic kits 90-A1 and 181-B). We assessed population differentials by determining the percent esterase-positive cells (macrophages) using standard light microscopy. Cells in the microtiter plates were stained with 0.4% crystal violet to determine survival (*11*).

***Assay of Dicoumarol-Inhibitable Quinone Reductase Activity.*** Quinone reductase (QR) activity was assayed by modification of the microtiter plate procedure developed by Prochaska and Santamaria (*25*). In this procedure, QR activity is assessed by measuring the dicoumarol-inhibitable NADPH-dependent menadiol-mediated reduction of 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to the blue formazan dye. For this assay we trypsinized stromal cells, spun them at 1000 rpm for 10 min, discarded the supernatant, and lysed the cells by incubation at 37°C for 10 min with 0.8% digitonin in 2 mM EDTA, pH 7.8. We assessed dicoumarol-inhibitable QR activity by adding the MTT reaction mixture, described below, to digitonin-lysed stromal cells in the presence and absence of 60 μM dicoumarol. We recorded the spectrophotometric kinetic measurement of change in absorbance/min at 610 nm and calculated QR activity using the extinction coefficient for MTT (11,300 $M^{-1}$ $cm^{-1}$) and expressed this value as nmol MTT reduced/min/mg protein. Protein was quantified by a modified Lowry assay (*26*) or by the Bio-Rad protein assay (cat. no. 500-0006), which is based on the Bradford assay (*27*). The MTT reaction mixture consisted of 25 mM Tris-Cl, 0.67 mg/ml bovine serum albumin, 0.01% Tween-20, 5 μM flavin adenine dinucleotide, 1 mM glucose-6-phosphate, 30 μM NADP, 2 units/ml glucose-6-phosphate dehydrogenase, 0.3 mg/ml MTT, and 50 μM menadione.

***Granulocyte/Monocyte Colony Formation in Soft Agar.*** We prepared concentrated 10X agar solution (3% w/v) by dissolving 3 g agar (Gibco cat. no. M00010) in 100 ml distilled water and sterilized it by autoclaving. For CFU-G/M assays, bone marrow cells were harvested into DMEM, with no additives, as described previously, centrifuged, and nucleated cells were counted by Coulter counter using a red cell lysing agent, "zapaglobin." We heated sterile 10X agar to boiling and diluted it 1:10 ($C_f$ 0.3% agar) with 39°C DMEM supplemented with 15% FBS, 2 mM glutamine, 50 μM 2-mercaptoethanol, 100 μg/ml L-asparagin, 100 IU/ml

penicillin, and 100 µg/ml streptomycin. We then added cells to the agar mixture in no more than 100-µl volumes to produce nucleated cell concentrations ranging from 4 to 20 × $10^4$ cells/ml. Next we added 1 ml of the cell–agar suspension to gridded TC35 dishes containing 100 µl of medium containing colony-stimulating factor. The colony-stimulating activity (CSA) was derived from medium that had been conditioned (12 days) by primary bone marrow stromal cells in the presence or absence of hydroquinone and/or DTT, as described below. After plating the cell-agar mixture, we allowed the agar to solidify at room temperature and incubated cultures at 37°C in a humidified, 5% $CO_2$ atmosphere for 8–12 days. Colonies consisting of at least 50 cells were scored from four replicate plates per test group using an inverted phase-contrast microscope.

***Hydroquinone Inhibition of Stromal Cell Ability to Support Myelopoiesis in Soft Agar.*** The effect of noncytotoxic doses of hydroquinone on the ability of DBA/2-derived stromal cells to support myelopoiesis was evaluated by treating 10–14-day cultures with 15 µM HQ and allowing the treated cells to condition the media for 12 days. We then collected this conditioned media and concentrated it for use in the CFU-G/M assay described above. Because the HQ was not removed during the media-conditioning period, we also determined CFU-G/Ms with conditioned media from untreated cells and added 20 µM HQ at the time of the colony-forming assay to control for any effect that residual HQ may have had on myelopoiesis (data not shown). Stromal cell conditioned media was concentrated approximately 10-fold in CentriCells (Polysciences, Inc.) with a 30,000 molecular weight cut-off point. We centrifuged the CentriCells at 2000*g* in a swinging-bucket rotor for 30 min. After centrifugation, we determined the volume of the least concentrated sample and added DMEM with all additives to the other samples to bring them up to this volume so that all samples had an equal concentration. Individual CentriCells were used a maximum of 3 times, and concentrated volumes on any one run varied by ± 10%.

***In Vivo Feeding of DTT.*** We acclimated DBA/2 mice (9–10-week-old males) and maintained them on an antioxidant-free powdered diet (AIN-76 purified diet with additional menadione and without ethoxyquin, TEKLAD DIETS) for 1 week. The test group was switched to diet containing 0.1% DTT, a concentration which was well tolerated by the animals based on appearance, weight, and activity levels. After 6 days on test or control diet, animals were humanely euthanized by cervical dislocation, and bone marrow and livers were removed for enzyme analysis. We determined QR activity in whole bone marrow and in 24-hr primary cultures of bone marrow stromal cells. Livers were used as a positive control for QR and glutathione-*S*-transferase(GST) induction by DTT (data not shown).

In experiments to determine if *in vivo* feeding of 0.1% DTT could protect primary bone marrow cells against *ex vivo* challenge by hydroquinone, we treated animals as described immediately above and flushed bone marrow cells from the femurs using two mice per *n*. Equal numbers of nucleated cells were then plated into four TC100 dishes per *n* in the presence (two plates) or absence (two plates) of 50 µM HQ, the $LC_{50}$ dose for DBA/2 primary stromal cells. By this protocol each *n* had two test plates and two control plates to serve as its own control. Twenty-four hours later, we washed plates four times with PBS to remove dead and unattached cells. We then collected surviving, attached cells by scraping the plates with a rubber scraper and assessed survival by counting the number of surviving cells using a Coulter counter. Data are expressed as percent of survival for controls.

***Statistics.*** Computations and statistics were performed using Lotus and Statpak software on an IBM personal computer. We used Students *t*-test (two-tailed) and one-way analysis of variance (ANOVA); values were considered significantly different if $p \leq 0.05$ or if the *F* ratio had a significance ≤ 0.05.

## Results

Based on more extensive dose–response studies, the $LC_{50}$ for hydroquinone was determined to be 49 ± 6 µM for 3 × $10^4$ stromal cells/well for DBA/2 (*11*). Twenty-four hours of pretreatment of stromal cells with 75 µM DTT protected against HQ-induced cytotoxicity, even at 95 µM HQ, a concentration that killed all cells in non-DTT-treated controls (*12*).

When DBA/2-derived stromal cells were exposed to $LC_{25}$ and $LC_{50}$ concentrations of HQ and the proportion of macrophages versus fibroblasts to untreated cells were compared, the proportion of macrophages in the HQ-treated cells dropped from approximately 60% to about 40%, indicating that the macrophages were more sensitive to HQ than the fibroblasts (Table 1). This is consistent with results obtained by Thomas and co-workers with cells derived from $B6C3F_1$ mice (*6*). At the $LC_{25}$ of HQ, at which 25% of the cells are killed, a 20% decrease in the macrophage population means that virtually all the cells that were killed were macrophages. In the same experiment, another group of cells was pretreated for 24 hr with 75 µM DTT before exposure to HQ, and the percentage of macrophages versus fibroblasts was determined. DTT completely protected the cells from the cytotoxic effects of HQ, and the proportion of macrophages to fibroblasts was identical to control populations (Table 1). As previously reported, QR activity approximately doubles, and GSH concentration increases by about one-third after pretreatment with 75 µM DTT (*12*). The increases in both GSH concentration and QR activity would therefore appear to underlie the chemoprotective actions of DTT. Mechanistically, GSH would interact directly with the electrophilic benzoquinone, whereas increased QR activity would reduce benzoquinone to the less chemically reactive hydroquinone.

The studies described thus far used only cell death as the endpoint for toxicity to HQ. Because the stromal element of the bone marrow is involved in the regulation and maintenance of hematopoiesis, chemical toxicity could also be expressed by impairment of regulatory function without causing cell death. This form of HQ-induced toxicity has recently been reported in long-term stromal cultures derived from the $B6C3F_1$ mouse (*28*). Consequently, we were interested to see if DTT could protect primary stromal cells from HQ-induced toxicity using this functional endpoint as an assessment of toxicity. Stromal cell support of myelopoiesis can be assayed in two ways: by plating cells in semisolid agar directly onto adhered stromal cells or by plating cells in agar onto media that has been conditioned by stromal cells. Conditioned media has to be

**Table 1.** Effects of HQ on macrophage versus fibroblast differential in DBA/2 primary bone marrow stromal cells

| HQ concentration (µM) | Survival (%) | | Esterase-positive cells (%)[a] | |
|---|---|---|---|---|
| | -DDT | +DTT | -DTT | +DTT[b] |
| 0 | 100 | 100 | 59 ± 3[b] | 58 ± 2 |
| 35 | 72 ± 3 | 108 ± 1 | 38 ± 2* | 57 ± 1 (NS) |
| 50 | 53 ± 8 | 99 ± 5 | 40 ± 1* | 61 ± 2 (NS) |

Values are means ± SEM, with an $n \geq 3$; NS, not significantly different from untreated control (-DTT, 0 HQ) by one-way ANOVA, $F = 0.883$.
[a] Macrophages.
[b] Cells pretreated with 75 µM DTT 24 hr before HQ administration.
*Significantly different from untreated control (-DTT, 0 HQ) by one-way ANOVA, $F = 38.666$.

**Table 2.** DTT 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. of colonies[a] (mean ± SEM) |
|---|---|---|
| None | NA | 2 ± 1 |
| Stromal medium[b] | None | 361 ± 19 |
| Stromal medium | 15 μM HQ | 204 ± 21* |
| Stromal medium | 75 μM DTT pretreatment followed by 15 μM HQ | 367 ± 46 (NS) |

CSA, colony-stimulating activity; NA, not applicable; NS, not significantly different from number of colonies grown in the presence of medium conditioned by untreated cells, by Student's *t*-test. Stromal cultures were used to condition media for 12 days in the presence or absence of HQ and DTT.
[a] $3 \times 10^5$ white blood cells were plated per TC35 dish in this colony-forming assay.
[b] Conditioned medium was concentrated by centrifugation in Centricells to about 1/15 volume.
*Significantly different from number of colonies grown in the presence of medium conditioned by untreated cells; Student's *t*-test, $p<0.02$.

concentrated to express enough colony-stimulating activity to support myelopoiesis. We used the second method to obtain the stromal cell-derived colony-stimulating factors necessary for the support of myelopoiesis because conditioned media has the additional advantage of allowing us to treat the stromal cells without concurrently treating the naive bone marrow cells used in the colony-forming assay.

The data presented in Table 2 illustrate that 15 μM HQ, which is a noncytotoxic concentration for DBA/2-derived stromal cells, impaired the ability of stromal cells to support myelopoiesis as indicated by a reduction in the number of colonies compared to media obtained from untreated cells. Pretreatment of the stromal cells with 75 μM DTT 24 hr before exposure to 15 μM HQ protected the functional integrity of the stromal cells as indicated by the ability of the conditioned medium to support the growth of the same number of colonies as untreated stromal cells. To control for any effects that residual HQ or DTT in the concentrated, stromal cell-conditioned media may have had on colony formation, a second series of colony-forming experiments were performed. In the second set of experiments, 20 μM HQ or 75 μM DTT was added to the conditioned, concentrated media from untreated stromal cells at the time of assay for colony formation. Conditioned media from stromal cells treated with 20 μM HQ was also assayed in this experiment. Neither 20 μM HQ nor 75 μM DTT added at the time of assay had any effect on colony formation by naive bone marrow cells, and inhibition of stromal cell ability to support myelopoiesis by noncytotoxic concentrations of HQ was confirmed (data not shown).

The significant increase in QR activity resulting from DTT exposure focused our attention on this enzyme as a biomarker of DTT inductive chemoprotective effects in subsequent *in vivo* experiments. The final series of experiments were designed to test if the *in vivo* administration of DTT could induce QR activity in the bone marrow, and if so, could *in vivo* feeding of DTT protect bone marrow stromal cells from *ex vivo* challenge with HQ. It was necessary to remove the bone marrow cells from the animals for HQ challenge rather than challenge the animals *in vivo* to demonstrate definitively that relevant biochemical changes had occurred in the target organ. Demonstration of DTT protection against *in vivo* HQ or benzene challenge would require extensive pharmacokinetic analysis to establish if protection resides solely at the level of the bone marrow as opposed to (or in addition to) other sites such as the liver, as has been shown with oral benzo-[*a*]pyrene-induced bone marrow toxicity (*29*). *In vivo* feeding of DTT does induce QR activity in rat liver (*30*).

DBA/2 mice were fed 0.1% DTT mixed in the diet for 6 days. This dose of DTT was well tolerated as assessed by weight gain, activity levels, and overall appearance, which were comparable to control animals. At the end of the test period, we euthanized animals and cultured bone marrow cells for assessment of QR activity or treatment. Livers were also removed, perfused, and immediately frozen in liquid nitrogen to be used as a positive control, and 2- to 3-fold inductions of QR activity were observed (data not shown). Induction of hepatic QR activity was expected due to the fact that *in vivo* induction by a number of phase II inducers has been demonstrated in the DBA/2 mouse (*22*). As shown in Table 3, there was an increase in QR activity in endogenous bone marrow preparations as well as in 24-hr stromal cultures. DTT feeding increased QR activity in whole bone marrow preparations from 17 to 22 nmol/min/mg protein, and from 30 to 46 nmol/min/mg protein in 24-hr stromal cells (Table 3). In keeping with the induction of QR activity within the bone marrow compartment, *in vivo* feeding of DTT protected primary stromal cells against a subsequent *in vitro* challenge with 50 μM HQ during the first 24 hr in culture (Table 4).

## Discussion

The concept of chemoprotection, that is, protection from the toxicity of one chemical by the administration of another chemical, was first observed more than 50 years ago in rodents when skin tumors were produced by local application of carcinogens (*17,18*). Mechanistically, chemoprotection has been associated with the induction of three classes of detoxification enzymes: enzymes involved in ascorbic acid biosynthesis, the microsomal P450 class of enzymes, and soluble, cytosolic enzymes known collectively as phase II detoxification enzymes (*17,31*). Extensive research since that time has demonstrated that induction of phase II enzymes as well as

**Table 3.** Effect of *in vivo* feeding of DTT on quinone reductase activity in DBA/2-derived whole bone marrow and bone marrow stromal cells

| Cell preparation | Quinone reductase activity (nmol MTT reduced/min/mg protein) | |
|---|---|---|
| | Control | 0.1% DTT in diet |
| Whole bone marrow | 17 ± 1 | 22 ± 1* |
| Primary stromal cells[a] | 30 ± 2 | 46 ± 2* |

Values are means ± SEM, $n \geq 3$.
[a] Cells were allowed to adhere for 24 hr, washed repeatedly to remove nonadherent cells, then collected for assay.
*Significantly different from respective control by Student's *t*-test, $p<0.05$.

**Table 4.** *In vivo* feeding of DTT protects against *ex vivo* hydroquinone challenge in DBA/2-derived primary bone marrow stromal cells

| Hydroquinone concentration (μM) | Survival (%) | |
|---|---|---|
| | Control | 0.1% DTT in diet |
| 0 | 100 | 100 |
| 50 | 37 ± 4 | 62 ± 6* |

Animals were fed control or test diet for 6 days, and the bone marrow cells were flushed from femurs and plated into stromal media containing 0 or 50 μM HQ [2 animals/*n*, 2 plates/*n* (0 and 50 μM HQ), so that each *n* had its own control]; 24 hr later, cells were washed repeatedly, attached (live) stromal cells were collected by scraping and counted using a Coulter counter. Values are means ± SEM, $n = 4$.
*Significantly different from control survival by Student's *t*-test, $p \leq 0.02$.

glutathione is a useful strategy for enhancing the clearance and/or detoxification of chemically reactive intermediates (*14, 18,32*). Quinone reductase (QR) has also been shown to be coordinately induced with other electrophile-processing phase II enzymes such as glutathione-*S*-transferases (*14,17,18*). Recently, the protection of hepatoma cells against the toxicity of a number of redox-active xenobiotics has been linked to the *in vitro* induction of QR (*19*). A class of chemicals currently under extensive investigation as chemoprotective agents are the dithiolethiones (*33*).

Bone marrow is a target organ for toxicities induced by a spectrum of chemicals (*34*) including the environmental pollutants benzene and benzo[*a*]pyrene (BaP) (*35*). The stromal cell component from bone marrow is particularly susceptible to toxicity induced by several redox-active metabolites of the hematotoxin benzene, such as benzoquinone and HQ (*12,28,36*). Consequently, it was of great interest when recent studies in our laboratory demonstrated that bone marrow-derived stromal cells could also be protected against hydroquinone-induced cytotoxicity by pretreating cells with the phase II enzyme inducer DTT (*12*). This observation prompted us to further examine whether the inducing activity of DTT in bone marrow stroma also protects against HQ-induced modulation of stromal-dependent myelopoiesis. In this study, we have also examined whether *in vivo* feeding of DTT induces QR activity within the stromal compartment and as such protects against the *in vitro* toxicity of HQ.

As shown in Table 1, HQ was toxic to DBA/2-derived bone marrow stroma and demonstrated preferential killing of stromal macrophages. Primary cultures of bone marrow stroma are not a pure population of one type of cell; these cultures consist of a 60:40 mixture of resident macrophages and fibroblastoid cells, respectively (*24,37*). Primary stromal macrophages were more sensitive to HQ than the fibroblastoid stromal cells, as illustrated by the shift in the ratio of macrophages to fibroblasts from 60:40 to 40:60 after $LC_{25}$ or $LC_{50}$ doses of HQ (Table 1). Pretreatment of the stromal macrophages and fibroblasts with DTT prevented this shift in ratio (Table 1).

The data described above complement previous studies performed in our laboratory comparing QR and GST activities and GSH concentration between cell types within the bone marrow stroma and between strains of mice with differential susceptibility to HQ-induced cytotoxicity. These previous studies demonstrated that basal QR activity was lower in the stromal macrophage versus the stromal fibroblast, with activity for the mixed population falling between the values for the individual cell types (*12*). The lower QR activity in the more HQ-sensitive cell type mimicked the difference in QR activity previously observed between whole stromal populations from strains of mice with differential sensitivity to HQ-induced toxicity (*12*). These data also agree with previous data evaluating the effect of DTT on stromal cell QR and GST activities and cytosolic GSH concentration. Treatment of primary cells *in vitro* with 75 μM DTT resulted in a 2-fold increase in QR activity in DBA/2 stroma and about a one-third increase in cytosolic GSH concentration (*12*). Unlike other tissues (*22,32*), DTT treatment of primary bone marrow stromal cells had no inductive effect on GST activity. Thus, the increase of both GSH concentration and QR activity appear to be important in the chemoprotection against HQ-induced toxicity provided by DTT. This hypothesis was substantiated by the effects of dicoumarol, an inhibitor of QR activity (*31*), on HQ toxicity and through protection by DTT. Dicoumarol potentiated HQ toxicity and interfered with DTT protection against HQ-induced toxicity in stromal cells (*12,38*). Likewise, depletion of GSH in stromal cells by buthionine sulfoximine potentiated HQ-induced toxicity (*39*).

Because enhanced stromal cell survival was used as an index of chemoprotection from HQ-induced toxicity, we thought it was important to also examine a noncytotoxic functional endpoint of toxicity induced by HQ. A potential target for nonlethal toxicity is the ability of stromal cells to support myelopoiesis, which is an *in vivo* function of the stromal cells. It has been previously demonstrated that purified populations of bone marrow stromal fibroblasts can support myelopoiesis to only 50% of the number of colonies that are supported by mixed populations of stromal fibroblasts and stromal macrophages. Purified stromal macrophages cannot support significant myelopoiesis (*28*). The data presented in Table 2 demonstrate that noncytotoxic concentrations of HQ impaired the ability of stromal cells to support myelo-poiesis by approximately 40%. This indicated again that the stromal macrophage was the preferential target of noncytotoxic concentrations of HQ, with the amount of observed myelopoiesis being supported primarily by stromal fibroblasts. This is in agreement with the previous data demonstrating preferential killing of stromal macrophages over stromal fibroblasts by HQ (Table 1). Pretreatment of cells by DTT protected the primary stromal cells from both forms of HQ-induced toxicity (Tables 1 and 2).

Chemoprotection of primary bone marrow stromal cells by DTT was also observed after *in vivo* feeding of DTT. Using QR as a biomarker of the inducing effect of DTT within the bone marrow, a 1.5-fold increase in QR activity was observed in 24-hr stromal cultures derived from animals that had received 0.1% DTT in the diet for 6 days (Table 3). This inductive activity of DTT was reflected in protection against the cytotoxic effects of 50 μM HQ in stromal cells derived from DTT-treated animals compared to stromal cells derived from animals that did not receive DTT*in vivo* (Table 4). More importantly, based on the data presented in Table 2, a protective effect against HQ-induced alterations in myelopoiesis would also be expected. Thus, the inducibility of cellular defense mechanisms and xenobiotic-processing enzymes by chemoprotective agents such as DTT may prove to be a useful strategy in preventing chemically induced cell dysfunction and death in the bone marrow (Fig. 1). In this regard, one of the advantages of an agent like DTT is that it is a monofunctional inducer and as such does not require interaction with the Ah receptor for its inducing activity (*22*). The availability of chemicals that function as inducers primarily of phase II enzymes independent of the Ah receptor is important from the perspective that genetic differences in the *Ah* locus are relevant to humans as well as to different strains of mice (*21*).

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