Induced Differentiation of HL-60 Promyelocytic Leukemia Cells to Monocyte/Macrophages Is Inhibited by Hydroquinone, a Hematotoxic Metabolite of Benzene

By Nancy L. Oliveira and George F. Kalf

Chronic exposure of humans to benzene has been shown to have a cytotoxic effect on hematopoietic progenitor cells in intermediate stages of differentiation, which can lead to aplastic anemia and acute myelogenous leukemia. We studied the effect of hydroquinone (HQ), a toxic metabolite of benzene found in the bone marrow, on the human promyelocytic leukemia cell line (HL-60), which can be induced to differentiate to both monocyte and myeloid cells, and thus has been used as a surrogate for a granulocyte/macrophage progenitor cell. Exposure of HL-60 cells to noncytotoxic concentrations of HQ for 3 hours before induction with phorbol myristate acetate (TPA) caused a dose-dependent inhibition of the acquisition of characteristics of monocytic differentiation, such as adherence, nonspecific esterase (NSE) activity, and phagocytosis, but had no effect on cell proliferation. HQ appeared to be affecting maturation beyond the monoblast/promonocyte stages. HQ also prevented differentiation induced by 1,25-dihydroxyvitamin D$_3$ [1,25-(OH)$_2$D$_3$]; however, the block occurred after the acquisition of adherence. HQ at concentrations that inhibited monocytic differentiation had no effect on differentiation to granulocytes, suggesting that the block in the differentiation of these bipotential cells is a step unique to the monocytic pathway. HQ was unable to prevent differentiation induced by the macrophage-derived cytokine, interleukin (IL)-1, a differentiation factor for cells of the monocytic lineage.

Benzene is a heavily used industrial chemical, a ubiquitous environmental pollutant and a hematotoxic toxin and carcinogen. Chronic exposure of humans to benzene causes bone marrow depression, which can lead to aplastic anemia and acute myelogenous leukemia and some of its variant forms. Benzene hematotoxicity occurs when the hepatic metabolites phenol, catechol, and hydroquinone (HQ) are transported to the bone marrow and are further oxidized by a peroxidase-mediated pathway to biologically reactive species that interfere with hematopoiesis. Benzene has a cytotoxic effect on hematopoietic progenitor cells in intermediate stages of differentiation, but it is not known whether HQ toxicity causes a lack of growth factors required for progenitor cell survival and differentiation or whether HQ has a direct effect on the differentiation of progenitor cells of the monocytic lineage.

We studied the effect of HQ on the differentiation of the human promyelocytic cell line HL-60, which has been used as a surrogate for a granulocyte/macrophage progenitor cell. These bipotential cells can be induced to differentiate into functional monocytes/macrophages by agents such as phorbol myristate acetate (TPA) or 1α,25-dihydroxyvitamin D$_3$ [1,25-(OH)$_2$D$_3$]. Treatment with retinoic acid or dimethyl sulfoxide (DMSO) induces them to differentiate to granulocytes. We report here that exposure of HL-60 promyelocytic cells to noncytotoxic concentrations of HQ causes a dose-dependent inhibition of differentiation to monocyte/macrophages, but does not affect differentiation to granulocytes.

MATERIALS AND METHODS

Cell culture. HL-60 human promyelocytic leukemia cells were obtained from the American Type Culture Collection (ATCC no. 240-CCL; Rockville, MD). Cells were cultured at 37°C in an atmosphere of 5% CO$_2$ in RPMI 1640 medium (Mediatech, Washington, DC) supplemented with penicillin (50 IU/mL) (Mediatech), streptomycin (50 mg/mL) (Mediatech), and 10% fetal bovine serum (FBS; Sigma, St Louis, MO). Cells used in experiments were from passages 18 to 42. The cells were free of mycoplasma, as determined by periodically testing the culture supernatant with a radiolabeled mycoplasma cDNA (Gen Probe test kit; San Diego, CA).

Treatment of cells with HQ and inducing agents. Noncytotoxic levels of HQ were determined by incubating the cells (5 × 10$^6$ cells/mL) with concentrations of HQ from 0.01 μmol/L to 10 μmol/L for 4 hours in Dulbecco's phosphate-buffered salt solution (PBS) containing 2 mg/mL glucose (Sigma) at 37°C, 5% CO$_2$. The cells were washed with PBS twice, resuspended in RPMI 1640 with 10% FBS, and incubated for 24 hours, after which cell viability was determined by trypan blue exclusion.

To test the effect of HQ on the induction of differentiation, cells (5 × 10$^6$/mL) were exposed to noncytotoxic levels of HQ in PBS containing 2 mg/mL glucose at 37°C for periods ranging from 24 minutes to 6 hours, depending on the experiment. Cells were carefully washed with PBS to flush out all HQ not covalently bound during the incubation, resuspended in RPMI 1640 with 10% FBS, and induced to differentiate.

For induction of monocytic differentiation, HQ-treated cells (5 × 10$^6$/mL) were cultured in the presence of either 20 nmol/L TPA, 125 nmol/L 1,25-(OH)$_2$D$_3$, or 100 U/mL recombinant human interleukin (IL)-1β for 4 days at 37°C. For induction of granulo-
cytic differentiation, cells were cultured with either 1 μmol/L retinoic acid or 1.25% DMSO for 7 days.

Assessment of differentiation. Differentiation of monocytes/macrophages was assessed by the acquisition of the following characteristics: adherence to plastic; nonspecific esterase (NSE) activity measured with α-naphthyl acetate (Sigma) according to the method of Yam et al.; the ability to phagocytize opsonized sheep red blood cells; the presence of monocyte-specific surface antigen Mo2 as determined by fluorescent microscopy using an Mo2-specific monoclonal antibody; and morphology. In brief, adherence was measured by gently washing off nonadherent cells, spinning them down, and counting them. This count was then subtracted from the starting count (5 × 10^6 cells/mL) and adherent percentage calculated. NSE activity was determined by incubating the cells with α-naphthyl acetate and hexazonium pararosaniline (Sigma) in 0.1 mol/L sodium phosphate buffer, pH 7.4, for 5 minutes, then counting the number of positive (ie, essentially black cells) and negative cells, and calculating the percentage. Negative controls were first incubated with 2% NaF to inhibit NSE activity. Phagocytosis was determined by incubating the cells with opsonized sheep red blood cells (Rockland, Gilbertsville, PA) for 2 hours at 37°C. Smears were then stained with Giemsa stain and the cells examined for the presence of phagocytized blood cells (three or more engulfed blood cells constituted a positive cell). Morphological assessment of differentiation was performed by fixing cells to glass slides with Cytofix fixative (Shandon, Pittsburgh, PA), followed by automated Wright-Giemsa staining. Percentages were based on the average obtained by counting 300 cells on each of triplicate slides.

Differentiation to granulocytes was determined by the acquisition of the following characteristics: development in nonadherent cells of superoxide production, detected by the reduction of nitroblue tetrazolium (NBT); and the presence of a granulocyte-specific surface antigen recognized by the monoclonal antibody L12-2 (the kind gift of Dr Giovanni Rovera, Wistar Institute, Philadelphia, PA) using fluorescent microscopy. Briefly, cells were incubated for 1 hour with 0.125 nmol/L TPA and stained with safranin and cells were examined for the presence of black granules. Morphological assessment of differentiation to granulocytes was as previously described for monocytes. The presence of the L12-2 surface antigen was detected using rhodamine-labeled antimouse immunoglobulin (Sigma) against the monoclonal antibody.

Determination of cellular proliferation. Cellular proliferation was measured by the incorporation of [3H]thymidine into DNA. After exposing cells to HQ and/or TPA in the presence of [methyl-3H]thymidine (specific activity, 82 Ci/mmol, 5 μCi/culture) (Amersham, Arlington Heights, IL), cells were lysed and the trichloroacetic acid (TCA)-precipitable material was collected on filter disks, which were washed in TCA followed by ethanol and dried. The disks were placed in vials and the DNA was solubilized by a 15-minute exposure to Protosol (0.5 mL; Dupont/NEN, Boston, MA). Formula 949 (Dupont/NEN) scintillator was added to the vials and the samples were counted in the liquid scintillation spectrometer.

Determination of myeloperoxidase activity. Myeloperoxidase activity was determined by the method of Himmelhoch et al. Briefly, an incubation mixture consisting of 500 μL 200-mmol/L potassium phosphate buffer, pH 7.0, 250 μL H₂O₂, 100 μL cell lysate, 100 μL 0.13-mol/L guaiacol (Sigma), and 50 μL 10-mmol/L H₂O₂ was monitored for absorbance at 470 nm over a 2-minute period.

**RESULTS**

Effect of HQ on differentiation of HL-60 cells to monocyte/macrophages induced by TPA or 1,25-(OH)₂D₃. Incubation of HL-60 cells in the presence of TPA results in their differentiation into monocyte/macrophages (Fig 1A). Proliferation, as measured by [3H]thymidine incorporation into DNA, stops 12 to 14 hours after exposure to the inducer, and the cells begin to acquire more mature characteristics. The ability of the cells to adhere occurs immediately after the cessation of proliferation, whereas the ability to carry out phagocytosis is not acquired until 4 days after exposure to TPA (Fig 1A). Preincubation of the cells with nontoxic concentrations of HQ for 4 hours followed by removal of the HQ by washing of the cells before treatment with TPA prevents the development of adherence and phagocytosis, but has no effect on the level of cellular proliferation in HL-60 cells or on the cessation of proliferation induced by TPA (Fig 1B). The ability of HQ to prevent TPA-induced differentiation required a minimum of 3 hours of exposure (Fig 2). Exposures of 4 and 5 hours showed similar results; consequently, a 4-hour exposure was used in all

![Fig 1](image-url)
HYDROQUINONE INHIBITS HL-60 CLL MONOCYTOPOIESIS

for 2, 2.5, or 3 hours. TPA-treated control. The control values are 18% for 2 hours, 17.7% for 2.5 hours, and 26% for 3 hours. ***Significant difference at P < .001. Data are representative of two separate experiments.

Fig 2. Inhibition of TPA-induced differentiation of HL-60 cells to monocyte/macrophages as a function of time of exposure to HQ. Cells were incubated with 1 µmol/L HQ in PBS with 2 mg/mL glucose for 2, 2.5, or 3 hours. (□), TPA; (■), HQ/TPA. Bars represent the mean ± SD of triplicate samples, expressed as percent of the TPA-treated control. The control values are 18% for 2 hours, 17.7% for 2.5 hours, and 26% for 3 hours. ***Significant difference at P < .001. Data are representative of two separate experiments.

subsequent experiments. Inhibition was a function of the concentration over a range of 0.1 to 5 µmol/L (Fig 3). These concentrations have no effect on cell viability (data not shown). In the absence of TPA, HQ had no effect on parameters indicative of differentiation.

The degree of differentiation induced by TPA and the effect of HQ on the process in a typical morphology experiment are presented in Table 1. These data show that while TPA induces differentiation of HL-60 cells (colony-forming unit–granulocyte-monocyte [CFU-GM])²⁹ mainly to mature monocyte/macrophages, a significant percentage of monoblasts and promonocytes (intermediate cell types) are also found. HQ appears to affect maturation of monoblasts and promonocytes to more mature cells.

HQ also affects the differentiation caused by the physiological inducer, 1,25-(OH)₂D₃ (Fig 4). Inhibition of 1,25-(OH)₂D₃-induced NSE activity, phagocytosis, and the monocyte-specific surface antigen Mo2 occurred, but the development of adherence was not significantly affected.

Fig 3. Inhibition of TPA-induced differentiation of HL-60 cells to monocyte/macrophages as a function of HQ concentration. Cells were incubated with 0.1, 1, or 5 µmol/L HQ in PBS with 2 mg/mL glucose for 4 hours. (□), Untreated; (●), 1 µmol/L HQ; (■), TPA; (□), 0.1 µmol/L HQ/TPA; (■), 1 µmol/L HQ/TPA; (□), 5 µmol/L HQ/TPA. Bars represent the mean ± SD of triplicate samples, expressed as percent of the TPA-treated control. The control values are 53% for adherence, 45% for NSE, and 51% for phagocytosis. ***Significant difference at P < .001. Data are representative of three separate experiments.

Table 1. Effect of HQ on Differentiation of HL-60 Cells to Monocyte/Macrophages

<table>
<thead>
<tr>
<th>System</th>
<th>Cell Type (% of total)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HL-60</td>
</tr>
<tr>
<td>HQ/1,25(OH)₂D₃</td>
<td>60.3 ± 1.5</td>
</tr>
<tr>
<td>IL-1</td>
<td>60.3 ± 1.5</td>
</tr>
<tr>
<td>HQ/IL-1</td>
<td>60.3 ± 1.5</td>
</tr>
</tbody>
</table>

Values represent the mean percentage ± SD of the differentiation of cells obtained by counting 300 cells on each of triplicate slides. Smears were stained with Wright-Giemsa stain using automated methods.

*Intermediate represents monoblasts and promonoblasts.
†Cells (5 x 10⁵/mL) were incubated in PBS with 2 mg/mL glucose for 4 hours, then cultured for 4 days in RPMI with 10% FBS.
‡Cells were incubated as described for †, with the addition of the appropriate inducer: TPA (20 nmol/L); 1,25-(OH)₂D₃ (120 nmol/L); recombinant human IL-1β (100 U/mL).
§Cells treated with HQ were incubated in PBS with 2 mg/mL glucose and 1 µmol/L HQ for 4 hours, then cultured for 4 days in RPMI with 10% FBS.

Fig 4. Inhibition of 1,25-(OH)₂D₃-induced differentiation of HL-60 cells to monocyte/macrophages. Cells (5 x 10⁵/mL) were incubated with 1 µmol/L HQ in PBS with 2 mg/mL glucose for 4 hours at 37°C, 5% CO₂, then washed and incubated with 125 nmol/L 1,25-(OH)₂D₃ for 4 days. (□), Untreated; (●), 1,25-(OH)₂D₃; (■), HQ/1,25-(OH)₂D₃ Bars represent the mean ± SD of quadruplicate samples, expressed as percent of the 1,25-(OH)₂D₃-treated control. The control values are 29.5% for adherence, 55.2% for NSE, 26.6% for phagocytosis, and 45% for Mo2 antigen. ***Significant difference at P < .001. Data are representative of three separate experiments.
Effect of HQ on differentiation of HL-60 cells to monocytes/macrophages induced by IL-1. Since IL-1 has been reported to be a differentiation factor for cells of the monocytic lineage, and since HQ inhibits the processing of pre-IL-1α to mature cytokine in stromal macrophages, we tested the ability of HQ to prevent IL-1-induced differentiation of HL-60 cells to monocyte/macrophages. Exposure of HL-60 cells to recombinant human IL-1β induced the appearance of NSE and phagocytosis, but did not cause the cells to adhere (Fig 5). IL-1 stimulated differentiation to monocytes when cells were grown in the presence of either 1% or 10% serum. HQ (1 μmol/L) had no effect on IL-1-induced differentiation to monocytes at 10% FBS (Fig 5) or at 1% FBS (data not shown).

Effect of HQ on differentiation of HL-60 cells to granulocytes. As shown in Table 2, treatment of HL-60 cells with 1 μmol/L retinoic acid resulted in their differentiation to granulocytes as evidenced by morphology, the development of the ability to produce superoxide (as monitored by the reduction of NBT), and the appearance of the granulocyte-specific surface antigen, L12-2. Exposure to 1 μmol/L HQ for 3 hours, which completely inhibits monocytic differentiation, had no effect on retinoic acid- or DMSO-induced differentiation to granulocytes (Table 2). Furthermore, concentrations of HQ up to 5 μmol/L with exposure for as long as 6 hours also had no effect, either on retinoic acid- or DMSO-induced differentiation to granulocytes or cell viability (data not shown).

Activation of HQ to reactive species in HL-60 cells. The ability of HQ to inhibit differentiation in HL-60 cells is undoubtedly dependent on its activation in the cell to 1,4-benzoquinone, which covalently binds to cellular macromolecules and is known to affect cellular functions. Oxidation of HQ to 1,4-benzoquinone occurs by a peroxidase-mediated reaction. Promyelocytes contain myeloperoxidase, therefore, HL-60 cells were assayed for myeloperoxidase activity and its ability to activate [14C]HQ to species that could covalently bind to cellular proteins and result in an inhibition of differentiation. HL-60 cells were shown to contain 0.148 guaiacol units of myeloperoxidase activity per 10^6 cells. When these cells were incubated with [14C]HQ (0.1 μCi/10^6 cells), 45 pmol of [14C]HQ equivalents (1.4-benzoquinone) was bound to macromolecules measured as TCA-precipitable radioactivity not extractable by organic solvents. These results indicate that HL-60 cells have the capacity to convert HQ to a reactive molecule with the potential to inhibit monocytic differentiation.

DISCUSSION

Exposure of HL-60 cells to noncytotoxic concentrations of HQ for 3 hours before induction of differentiation with TPA causes a dose-dependent inhibition of the acquisition of characteristics of monocytic differentiation such as morphology, adherence, NSE activity, and phagocytosis (Fig 3, Table 1). The concentration of HQ (1 μmol/L) that inhibited the acquisition of these monocytic characteristics is 100 times less than the concentration detected in the bone marrow of experimental animals following a dose of benzene that causes significant bone marrow depression. Under the conditions used, HQ has no effect on the proliferation of HL-60 cells (Fig 1B) and does not prevent the inhibition of cell proliferation observed after the addition of the inducer. Cells treated with noncytotoxic doses of HQ in the absence of inducer showed properties similar to control cells. In the presence of TPA, cells progress from the CFU-GM stage of untreated HL-60 cells, to intermediate monoblast/promonocyte stages, and then to monocytic/macrophages. HQ appears to mainly affect maturation beyond the intermediate monoblast/promonocyte stages (Table 1).

HQ also prevents differentiation of HL-60 cells to monocyte/macrophages induced by 1,25-(OH),D₃, a physiological inducer. In this case, the block to differentiation occurred after the acquisition of adherence, which in the case of TPA-induced cells appears within 24 hours after exposure (Fig 1), but before the appearance of later phenotypic changes, which generally occur 3 to 4 days after exposure to the inducing agent (Fig 1). The development of NSE activity and phagocytosis, and the appearance of the monocytic-specific membrane antigen Mo2, were significantly inhibited by HQ (Fig 4).

The ability of HQ to inhibit differentiation is restricted to the cells of the monocytic lineage. HQ, at concentrations fivefold higher than those that inhibit monocytic differentiation, has no effect even with prolonged exposure on induction of differentiation to granulocytes by either DMSO or retinoic acid, as measured by morphology, reduction of NBT, and the acquisition of the granulocyte-specific surface antigen L12-2 (Table 2). The block in the differentiation of HL-60 cells appears to occur at the intermediate monoblast/promonocyte stage.

The mechanism whereby HQ specifically prevents monocytic differentiation is not known. Several possibilities can be ruled out based on the timing of the HQ treatment.

Fig 5. Inability of HQ to prevent IL-1-induced differentiation of HL-60 cells to monocyte/macrophages. Cells (5 x 10⁶/mL) were incubated with 1 μmol/L HQ in PBS with 2 mg/mL glucose for 4 hours at 37°C, 5% CO₂, then washed and incubated with 100 U/mL recombinant human IL-1β for 4 days. Untreated, (□), IL-1, (■), HQ/IL-1. Bars represent the mean ± SD of quadruplicate samples, expressed as percent of the IL-1-treated control. Control values are 34% for NSE and 42% for phagocytosis. Data are representative of two separate experiments.
Because the HL-60 cells are treated with and washed free of HQ before the addition of inducing agent, HQ is reacting with some essential component present in the HL-60 cells that is required for differentiation to occur once induction is initiated, rather than with some component produced in response to the inducer. For example, a direct interaction of HQ with macrophage colony-stimulating factor (M-CSF) or its receptor, c-fms, which are required for the CFU-GM cell to differentiate into a mature monocyte, is not possible because uninduced HL-60 cells do not express significant M-CSF mRNA until at least 8 hours of treatment with TPA or with granulocyte-macrophage CSF (GM-CSF), and no c-fms RNA is detected until at least 12 hours of treatment with TPA, 1,25-(OH),D, or rhGM-CSF. Similarly, differentiation of HL-60 cells to monocytes/macrophages requires coordinate and selective regulation of eicosanoid synthesis. Within 1 hour of the addition of TPA to HL-60 cells, a large increase in thromboxane B2 synthesis occurs that requires both activation of protein kinase C and induction of enzymes of the prostaglandin pathway such as thromboxane synthase, prostaglandin H synthase, and prostacyclin synthase. Here, also, HQ cannot be inhibiting either the induction of the enzymes or their activity, because it is not present during induction by TPA. In addition, benzene stimulates rather than inhibits prostaglandin synthesis in marrow stromal macrophages in vivo.

Another possibility is that HQ is inhibiting protein kinase C, which mediates induction of differentiation by both TPA and 1,25-(OH),D,. HQ does not prevent differentiation to granulocytes induced by DMSO and retinoic acid, both of which have been reported to exert their effects through protein kinase C even at concentrations fivefold higher than required to inhibit differentiation to monocytes. In addition, we have demonstrated in a preliminary experiment (December 1990) that concentrations of HQ higher than those used here do not prevent protein kinase C-induced phosphorylation of macrophage proteins.

Another possibility is that HQ has an effect on IL-1 production. 1,25-(OH),D, has been shown to synergize with interferon γ in the induction and secretion of IL-1 by HL-60 cells and has been shown to enhance the lipopolysaccharide-stimulated IL-1β gene transcription in the human promonocytic cell line U937. The amount of 1,25-(OH),D, that causes a significant increase in IL-1 production is a function of the concentration of serum in the culture medium, since approximately 99% of the vitamin D metabolites in serum are bound to a vitamin D-binding protein.

In 1% FBS, a twofold increase in IL-1 production was apparent at 0.1 nmol/L 1,25-(OH),D,. We have demonstrated that IL-1 induces differentiation of HL-60 cells grown in 10% serum, as measured by NSE and phagocytosis. Our results differ from those of Silvennoinen and Hurme, who reported that IL-1 had no effect on proliferation of HL-60 cells cultured in 10% FBS, but in medium containing 1% FBS the cells differentiated to monocytes and expressed increased IL-1 receptors on the cell surface. However, the presence of exogenous IL-1 suppressed monocytic differentiation. In our experiments, no difference was observed in the IL-1 effect in 1% or 10% FBS.

TPA has been shown to induce both IL-1β gene expression and differentiation of U937 cells to monocytes via the activation of protein kinase C. HQ, at concentrations that prevent differentiation induced by TPA and 1,25-(OH),D,, has previously been shown in our laboratory to prevent the processing of pre-IL-1α to the mature cytokine in mouse stromal macrophages induced to synthesize IL-1 with lipopolysaccharide. In HL-60 cells, concentrations of HQ that prevent differentiation induced by TPA or 1,25-(OH),D, do not affect differentiation induced by IL-1, suggesting that exogenously added IL-1 might be bypassing a block in the processing of IL-1 during differentiation to monocytes. For these reasons, we tested the ability of HL-60 cells to synthesize IL-1 and the effect of HQ on both its synthesis and processing. Under conditions where TPA or 1,25-(OH),D, induce differentiation, no production of either pre-IL-1 or mature cytokine was demonstrated by Western blot analysis of lysates of either untreated HL-60 cells or cells exposed to inducer over a 72-hour period (data not shown).

In summary, treatment of HL-60 cells with HQ before induction of differentiation prevents the acquisition of the monocytic phenotype induced by TPA or 1,25-(OH),D, by an unknown mechanism that appears to be specific for the monocytic pathway, because HQ does not affect differentiation to granulocytes induced by DMSO or retinoic acid. These results are of considerable significance for benzene hematotoxicity, because they demonstrate that the putative toxic metabolite, HQ, found in the bone marrow, is capable of preventing monocytopoiesis and thus the development of

### Table 2. Effect of HQ on Differentiation of HL-60 Cells to Granulocytes

<table>
<thead>
<tr>
<th></th>
<th>%NBT Reduction</th>
<th>%L-12-2 Antigen</th>
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<tbody>
<tr>
<td>Con*</td>
<td>17.3 ± 8.6</td>
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<td>92.4 ± 2.9</td>
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<td>RA†</td>
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<tr>
<td>HQ/RAT</td>
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</tr>
<tr>
<td>DMSOT</td>
<td>38.3 ± 4.2</td>
<td>ND</td>
<td>35.5 ± 4.6</td>
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<tr>
<td>HQ/DMSOT†</td>
<td>38.7 ± 2.3</td>
<td>ND</td>
<td>32.9 ± 4.2</td>
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</table>

Values represent the mean percentage ± SD of the cells obtained by counting 200 cells on each of triplicate slides for each assay.

*Cells (6 x 10⁶/mL) were incubated in PBS with 2 mg/mL glucose for 4 hours, then cultured for 7 days in RPMI with 10% FBS.
†Cells were incubated as described for *, with the addition 1 µmol/L RA or 1.25% DMSO.
‡Cells were incubated in PBS with 2 mg/mL glucose and 1 µmol/L HQ for 4 hours, then cultured for 7 days with the appropriate inducer.
the stromal macrophage, a target of benzene toxicity. The stromal macrophage is involved in hematopoietic regulation through the synthesis of several cytokines, including IL-1, which is involved in (1) the development of the pluripotent stem cell to the myeloid and lymphoid stem cell; (2) lymphocyte development; and (3) induction of cytokine production in stromal fibroblasts and endothelial cells. Since monocyte dysfunction and deficient IL-1 production have been implicated in aplastic anemia,7,8 the ability of HQ to inhibit both monocytopoiesis and IL-1 processing9 may bear heavily on its ability to cause aplastic anemia.

REFERENCES


Induced differentiation of HL-60 promyelocytic leukemia cells to monocyte/macrophages is inhibited by hydroquinone, a hematotoxic metabolite of benzene

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