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Hydroquinone, a benzene metabolite, increases the level of aneusomy of chromosomes 7 and 8 in human CD34-positive blood progenitor cells

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Benzene is an established human carcinogen, producing leukemia, hematotoxicity and perhaps lymphoma. Its carcinogenicity is most likely dependent upon its conversion to phenol and hydroquinone, the latter being oxidized to the highly toxic 1,4-benzoquinone in the bone marrow. Exposure of human lymphocytes and cell lines to hydroquinone has previously been shown to cause various forms of genetic damage, including aneusomy and the loss and gain of chromosomes. However, the target cells for leukemogenesis are the pluripotent stem cells or early progenitor cells which carry the CD34 antigen ($CD34^+$ cells). In this study, human cord blood, which is particularly rich in $CD34^+$ cells, was exposed to hydroquinone for 72 h in a medium that favored $CD34^+$ cell survival and growth. $CD34^+$ and $CD34^-$ cells were then isolated. Fluorescence *in situ* hybridization was employed to determine the level of aneusomy of chromosomes 7 and 8 in both cell types. $CD34^+$ cells were generally more susceptible to aneusomy induction by hydroquinone than $CD34^-$ cells. Increased trisomy and monosomy of chromosomes 7 and 8 were observed in $CD34^+$ cells ($P_{trend} < 0.001$), whereas in $CD34^-$ cells only an increased level of monosomy 7 was detected ($P_{trend} = 0.002$). Particularly striking effects of hydroquinone were observed in $CD34^+$ cells on monosomy 7 and trisomy 8, two common clonal aberrations found in myeloid leukemias, suggesting that these aneusomies produced by hydroquinone in $CD34^+$ cells play a role in benzene-induced leukemogenesis.

Introduction

Benzene is an important industrial chemical (>2 billion pounds produced annually in the USA alone) and a component of gasoline (1). It is an established cause of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) and may cause lymphocytic leukemias and non-Hodgkin's lymphoma in humans (2,3). The precise mechanism by which benzene induces these effects remains unclear, but its conversion to metabolites is essential (4). Benzene is metabolized in the liver by cytochrome P4502E1 (CYP2E1) to benzene oxide, which rearranges non-enzymatically to phenol. Phenol can either be conjugated to a sulfate or glucuronide or be hydroxylated to catechol, hydroquinone and 1,2,4-benzenetriol. It is believed that these polyphenolic metabolites then travel to the bone marrow, where they are oxidized to highly toxic quinones by myeloperoxidase and produce hematotoxic and leukemogenic effects (4–6). The conversion of hydroquinone to 1,4-benzoquinone is thought to be the major pathway leading to toxicity of benzene (4,7).

Phenol and hydroquinone are also common dietary constituents and by-products of the breakdown of protein and the glycoside arbutin, respectively, in the intestine (8,9). Levels of human intake of these compounds are therefore considerable and highly variable. Interestingly, high levels of blood protein adducts of quinones derived from phenol and hydroquinone have been detected in normal individuals (10), suggesting that these quinones may play a role in idiopathic MDS and certain forms of leukemia as well as in benzene-induced leukemia (4).

Aneusomy, the loss or gain of specific chromosomes, is common in MDS and leukemia. Monosomy 7 is the most common early event in therapy-related MDS and AML (11,12) and predisposes infants to juvenile AML (13,14). Trisomy 8 is also a common change detected in AML and has been associated with smoking-induced leukemia (15), which is thought to be due, in part, to the benzene content of cigarette smoke. Increased levels of monosomy 7 and trisomy 8 have been detected independently in the peripheral blood cells of benzene-exposed workers (16,17). The ability of benzene exposure to induce aneusomy of these chromosomes could therefore be important in the development of MDS and AML.

We have previously used fluorescence *in situ* hybridization (FISH) to show that hydroquinone and benzenetriol induce aneusomy of chromosomes 7 and 9 in the human HL60 cell line (18), and the loss and long-arm deletion of chromosomes 5 and 7 in cultured human lymphocytes (19). Eastmond and colleagues (20) have also observed similar effects in human lymphocytes. Terminally differentiated lymphocytes, however, are clearly not the critical target cells for benzene's leukemia-inducing effects in humans. Leukemia is thought to arise as a result of genotoxic damage to pluripotent stem cells or early progenitor cells in the bone marrow (21). These cells carry the CD34 surface antigen and can be isolated using this surface marker. Human cord blood is particularly rich in CD34-positive ($CD34^+$) cells (22). Here, we have used FISH to investigate the ability of hydroquinone to induce aneusomy of chromosomes 7 and 8 in isolated $CD34^+$ cells *in vitro* and have compared the effects in these target cells with those in terminally differentiated $CD34^-$ cells. We show that $CD34^+$ cells are highly sensitive to the aneusomy-inducing effects of hydroquinone.

Abbreviations: AML, acute myeloid leukemia; FISH, fluorescence *in situ* hybridization; MDS, myelodysplastic syndromes.

Materials and methods

Cord blood samples

Human umbilical cord blood samples were used as sources for hematopoietic progenitor cells and were obtained anonymously as discarded specimens from healthy, spontaneous, full-term deliveries. Institutional Review Board and Scientific Committee approval for sample collections was obtained from

all participating institutions. Human subject protocols were approved at investigating institutions.

Blood cell culture

Cord blood was collected in a sterile manner using 250 ml USP/CPD (citrate–phosphate–dextrose solution) blood pack units with a single integral donor tube 16-gauge, ultra-thin wall needle (Code 4R0837; Baxter Healthcare Products, Deerfield, IL) at room temperature. Within 12 h, the samples were transported to our laboratory in brown paper bags to prevent light exposure, and the cord blood was immediately processed. For each culture, whole cord blood (5 ml) was added to 45 ml of culture medium, composed primarily of 50% Chang Medium BMC and 50% Chang Medium PB (Irvine Scientific, Santa Ana, CA). Together, the culture medium consisted of 85% RPMI with L-glutamine, 20% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.75% phytohemagglutinin A, and 5% Giant cell tumor-conditioned medium. In addition, various cytokines, namely IL-3 (20 ng/ml), IL-6 (20 ng/ml), thrombopoietin (10 ng/ml), human stem-cell factor (10 ng/ml) and FLT-3 ligand (20 ng/ml) (all from Stem Cell Technologies Inc., Vancouver, BC), were added to the medium to stimulate progenitor cell growth. All of the whole cord blood cultures were incubated at 37°C for 72 h in a 5% CO_2 atmosphere.

Hydroquinone treatment

Hydroquinone (99%; Aldrich, Milwaukee, WI) was dissolved in PBS (pH 7.4) immediately prior to treatment. Blood cells were treated 24 h after culture initiation at final concentrations of 0, 2, 10 and 50 μM hydroquinone in the complete medium. All treatments were performed in duplicate for each cord blood sample and harvested after 48 h of chemical exposure. Cytotoxicity of hydroquinone was assessed by measuring cell viability among at least 200 blood cells at each dose before cell separation using a whole-blood erythrocyte lysing kit (Leinco Technologies, Ballwin, MO). This kit was employed to gently lyse erythrocytes while maintaining the viability of unfixed cells. Briefly, 0.2 ml lysing working solution was added to 0.1 ml whole-blood culture medium. After incubation at room temperature for 10 min, the mixed solution was centrifuged for 5 min at 500 *g* and the cell pellet washed and re-centrifuged. The supernatant was decanted and the cells checked for viability after staining with 0.16% trypan blue diluted in PBS. Mean viabilities were 71% in untreated cells, 76% in cells treated with 2 μM hydroquinone, 69% with 10 μM hydroquinone and 66% with 50 μM hydroquinone. Although small, this decrease in cell viability with increasing hydroquinone concentrations was statistically significant ($P_{\text{trend}} = 0.002$), which indicates increased cytotoxicity in treated cord blood, especially at higher doses.

Cell separation from whole-blood culture

Mononuclear cells were isolated from cultures by Ficoll–Paque density gradient centrifugation (Pharmacia, Piscataway, NJ) at 1340 r.p.m. for 35 min. The interphase layer of mononuclear cells was removed and washed in PBS. Isolated mononuclear cells were further separated into $CD34^+$ and $CD34^-$ cells according to the detailed protocol below. Granulocytes in Ficoll–Paque density gradient above the red blood cells were also carefully isolated and then washed in PBS.

$CD34^+$ cells were obtained using MiniMACS separation columns and isolation kits (Miltenyi Biotec Inc., Auburn, CA). $CD34^-$ cells were collected after passing through the column. Technical procedures were performed according to manufacturer's instructions. In brief, cells were resuspended to 300 μl volume with degassed PBS with 0.5% bovine serum albumin and 2 mM EDTA at pH 7.2 and room temperature. The cells were labeled with a blocking reagent to inhibit non-specific or Fc-receptor binding of the CD34 Multisort Microbeads to non-target cells. Immediately after mixing, 100 μl of anti-$CD34^+$ antibody was added. After vortexing for 2 s, the cells were incubated for 20 min at 6–12°C. Cells were then washed twice. The remaining cell pellet was suspended to 400 μl with the wash solution. After addition of magnetic bead antibodies, the cell solution was mixed thoroughly and incubated for 30 min at 6–12°C. The cells were then washed twice, resuspended in a final volume of 500 μl, and passed through pre-rinsed pre-isolation filters directly into an MS+/RS+ column attached to a magnetic separator (all from Miltenyi Biotech Inc.). The columns were rinsed three times. The $CD34^+$ cells attached to the column/magnet were collected by flushing the column after it was removed from the magnetic separator. $CD34^+$ cells were then reapplied to a second pre-rinsed MS+/RS+ separation column to increase $CD34^+$ cell purity.

Flow cytometric analysis

Confirmation of the purity of the $CD34^+$ cell isolation was performed using flow cytometric analysis. Aliquots of $CD34^+$ and $CD34^-$ separated cells were stained with $CD34^+$ antibody ($CD34^+$ anti-HPCA; Becton Dickinson, Franklin Lakes, NJ). An aliquot of 20 μl of antibody was applied to ~10^6 cells in 50 μl of PBS with 0.1% azide. This cell suspension was vortexed for 30 s and then incubated in the dark for 30 min at 2–8°C. The cells were then rinsed twice with the PBS–0.1% sodium azide solution, fixed with 1% paraformaldehyde and analyzed by fluorescence-activated cell sorting (FACS) system (Becton Dickinson) for FITC (procedure as described by manufacturer's recommendation). FACS analysis showed that on seven trials there was a median $CD34^+$ purity of 68% (range 55–78%) after double isolation.

Preparation of FISH slides

After the $CD34^+$ and $CD34^-$ cells were isolated from cultured cord blood, they were placed in a hypotonic solution (0.075 M KCl) for 30 min at 37°C and fixed at least three times with Carnoy's solution (methanol:glacial acetic acid, 3:1). Fixed cells were dropped onto pre-labeled glass slides and allowed to air dry. These slides were then stored at room temperature for at least 2 weeks before FISH was performed, and the rest of the unused slides were stored at –20°C in a N_2 atmosphere until future use.

FISH

A dual-color hybridization of chromosomes 7 and 8 was performed in interphase cells of these two cell populations. Detailed procedures for FISH with repetitive DNA probes have been described previously (18,23). Briefly, a human centromeric probe specific for chromosome 7 (α-satellite) directly labeled with SpectrumGreen (Vysis Inc., Downers Grove, IL) and a digoxigenin-labeled centromeric probe for chromosome 8 (Oncor Inc., Gaithersburg, MD) were used. The chromosome probes were mixed with hybrid solution from Vysis. An automatic denaturation and hybridization procedure was performed using the HyBrite Denaturation/Hybridization system (Vysis). The mixed centromere probes were then applied to cellular DNA on slides. Slides were covered with glass slips, denatured at 76°C for 10 min and left in the Hybrite system at 37°C for 2–3 days in order to obtain optimal signals. These slides were then post-washed in 0.5× SSC for 5 min at 70°C and rinsed in phosphate buffer three times at room temperature.

Signal detection and amplification

Hybridization signals on chromosomes 7 and 8 were detected as green and red, respectively, in a detection solution with 20 μg/ml anti-digoxigenin (Boehringer Mannheim, Indianapolis, IN) and 20 μg/ml FITC–avidin (Vector, Burlingame, CA) in phosphate buffer for 30 min at 37°C. After the slides were washed three times for 2 min each in phosphate buffer, the nuclei of cells were counterstained with the blue fluorescent dye 4,6-diamino-2-phenylindole (DAPI; 0.1 μg/ml) (Sigma, St Louis, MO) prepared in a mounting medium (Vector, Burlingame, CA). The hybridization signals were viewed by a fluorescence microscope equipped with epifluorescent illumination and a 100× oil immersion lens. A triple-bandpass filter for DAPI/FITC/Texas Red (excitation at 405, 490 and 570 nm, respectively; emission at 460, 525 and 635 nm, respectively) was used. All the stained slides were randomized and coded prior to scoring. All scorable $CD34^+$ interphase cells on each slide were analyzed. One-thousand $CD34^-$ cells were analyzed per dose per experiment. Aneusomy was detected as the loss or gain of chromosomes 7 and 8 observed in interphase cells. The number of interphase nuclei with 0, 1, 2, 3, 4 and >5 spots per probe and the total number of scored cells were recorded. A cell with one signal on a chromosome indicated loss of that chromosome (monosomy), and three signals indicated the gain of that chromosome (trisomy). Four signals per chromosome indicated tetraploidy if another targeted chromosome also displayed four signals; if not, tetrasomy was inferred.

Statistical analysis

Logistic regression analysis was performed separately for each cell type and for each of the following outcomes: monosomy, trisomy and tetrasomy (all versus normal diploidy) of chromosomes 7 and 8. Each model includes hydroquinone dose levels (0, 2, 10 and 50 μM) as a simple linear variable so the statistical significance of trend could be assessed by cell type for each aneuploid outcome. Categorical variables were included in the regressions to adjust for variation in the rates of aneuploidy among donors (represented by experiment numbers, 1, 2 and 3, etc.). From these analyses, the associated *P*-values for trend (P_{trend}) are reported; $P_{trend} < 0.05$ implies that there is a statistically significant dose–response within the cell type. Figures 1–3 show dose–response curves through the original data points from all the experiments, as well as lines that connect the aneusomy rate averaged for each dose in both $CD34^+$ and $CD34^-$ cells. These logistic models were also applied to calculate *P*-values for dose-to-dose comparisons. For example, they were used to compare aneusomy rates among $CD34^+$ cells at specific doses of hydroquinone treatment versus that at 0 μM in untreated control cells.

Results

Cord blood samples from three individuals were cultured and exposed to different doses of hydroquinone in three separate

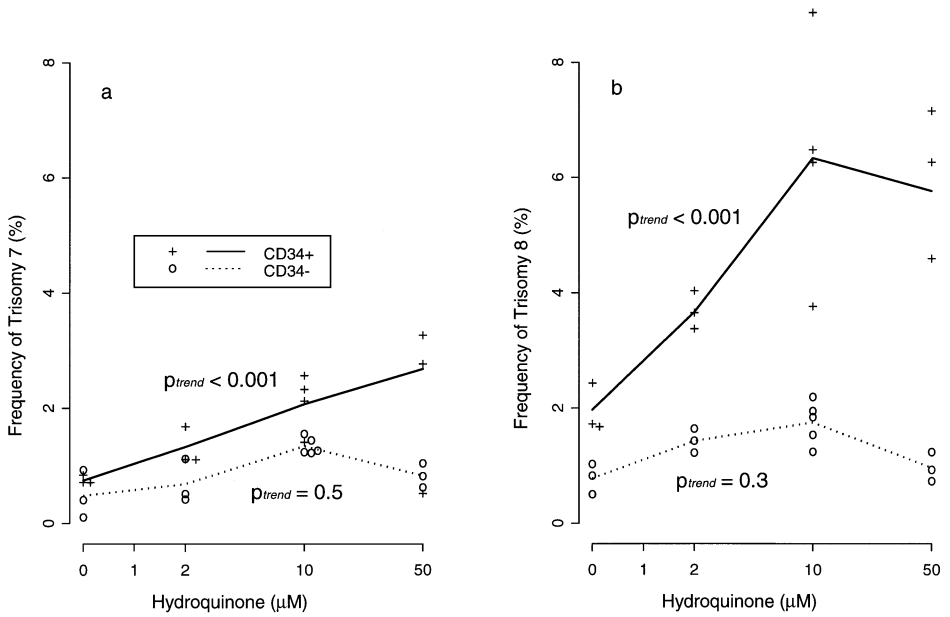

Fig. 1. Effect of hydroquinone on the frequency of trisomy of chromosomes 7 (**a**) and 8 (**b**) in $CD34^+$ and $CD34^-$ cells. Three different concentrations of hydroquinone were used: 2, 10 and 50 μM. The dose–response is shown in $CD34^+$ cells (+) and $CD34^-$ cells (○) with hydroquinone concentration plotted on a log-scale on the *x*-axis. Data from three to five individual experiments are shown with the plotted line connecting the means.

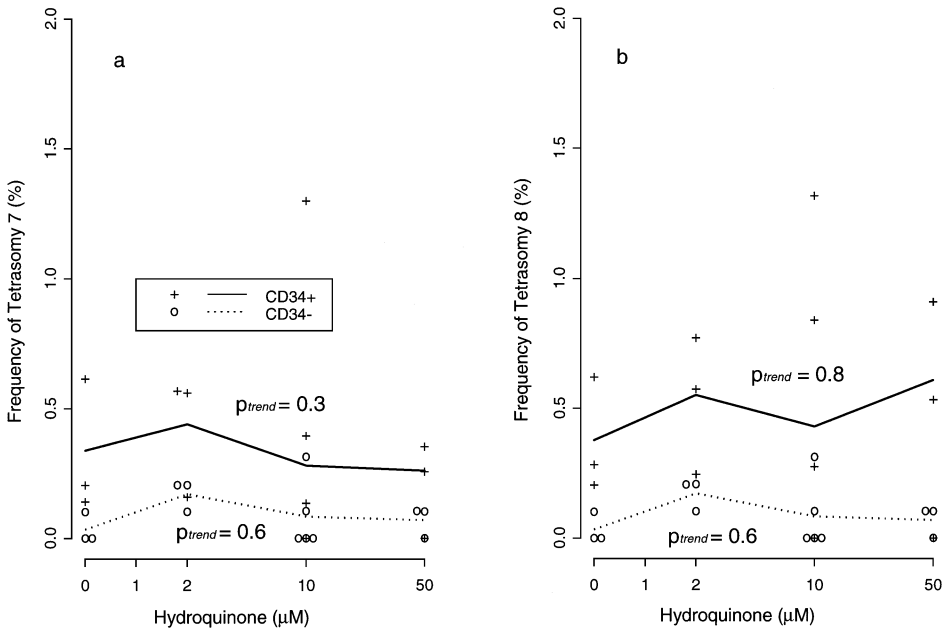

Fig. 2. Effect of hydroquinone on the frequency of tetrasomy of chromosomes 7 (**a**) and 8 (**b**) in $CD34^+$ and $CD34^-$ cells. Three different concentrations of hydroquinone were used: 2, 10 and 50 μM. The dose–response is shown in $CD34^+$ cells (+) and $CD34^-$ cells (○) with hydroquinone concentration plotted on a log-scale on the *x*-axis. Data from three to five individual experiments are shown with the plotted line connecting the means.

experiments (nos 1–3). $CD34^+$ and $CD34^-$ cells were isolated at the end of each experiment and analyzed by interphase FISH using centromere probes for chromosomes 7 and 8. The number of hybridization signals for each chromosome was determined in both cell types. In one experiment (no. 2), the slide(s) of $CD34^+$ cells after treatment with 10 μM hydroquinone did not hybridize well, making scoring impossible. Consequently, we performed two additional experiments (nos 4 and 5) at 10 μM hydroquinone and obtained data for $CD34^+$ and $CD34^-$ cells.

Cells with three hybridization signals of one color were considered to be trisomic for the labeled chromosome. Cells with four signals were considered tetrasomic. The effects of hydroquinone on the level of trisomy and tetrasomy of chromosomes 7 and 8 in the different cell types can be determined by viewing it graphically in Figures 1–3. These figures allow trends in the dose–response in the different cell types to be compared directly.

Effect of hydroquinone on levels of trisomy

Hydroquinone treatment produced significant dose-dependent increases in trisomy of chromosomes 7 and 8 in $CD34^+$ cells ($P_{\text{trend}} < 0.001$), but not in $CD34^-$ cells (Figure 1). Increases in trisomy 7 and 8 in treated $CD34^+$ cells were similar, ranging

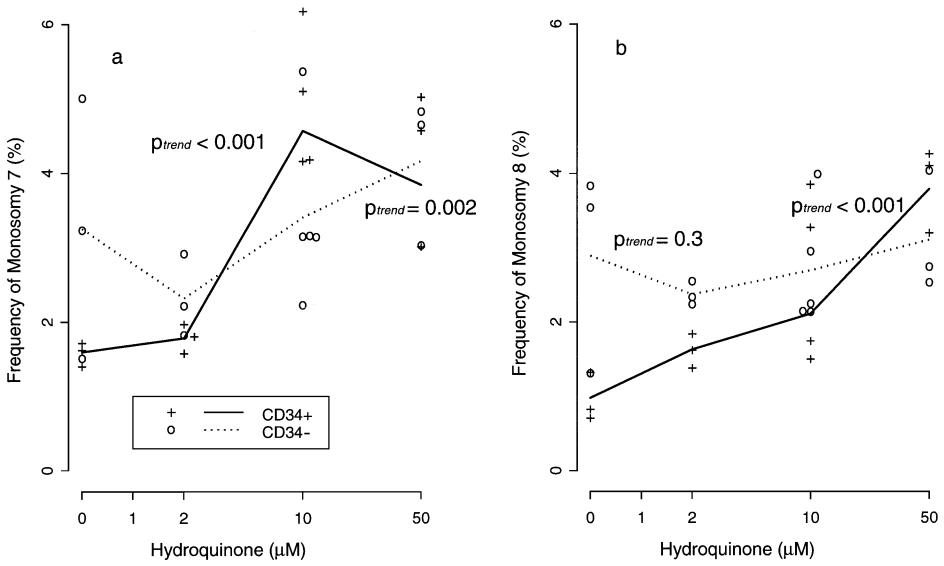

Fig. 3. Effect of hydroquinone on the frequency of monosomy of chromosomes 7 (**a**) and 8 (**b**) in $CD34^+$ and $CD34^-$ cells. Three different concentrations of hydroquinone were used: 2, 10 and 50 μM. The dose–response is shown in $CD34^+$ cells (+) and $CD34^-$ cells (○) with hydroquinone concentration plotted on a log-scale on the *x*-axis. Data from three to five individual experiments are shown with the plotted line connecting the means.

from 2- to 3-fold above background levels. The effects of hydroquinone on trisomy 8 may appear to be greater than trisomy 7 in $CD34^+$ cells (Figure 1b), but the high background level of trisomy 8 in these cells should be considered. Indeed, the dose-dependent increase in trisomy 8 was not statistically different from that in trisomy 7 in $CD34^+$ cells. The dose–response curves for trisomy 7 and 8 among $CD34^-$ cells and trisomy 8 among $CD34^+$ cells drops between 10 and 50 μM hydroquinone. This is probably due to increased cytotoxicity at 50 μM hydroquinone. Trisomy 8 frequencies were significantly increased in $CD34^+$ cells ($P < 0.001$) at concentrations of hydroquinone $\geqslant$2 μM over untreated control cells. The background level of trisomy 8 in untreated $CD34^+$ cells was also significantly higher than that in $CD34^-$ cells ($P < 0.001$). Since trisomy 8 is a common clonal aberration in AML, this finding suggests that induction of trisomy 8 in $CD34^+$ progenitor cells by hydroquinone may play a role in benzene-induced AML.

Effect of hydroquinone on levels of tetrasomy

The effects of hydroquinone treatment on tetrasomy 7 and 8 levels are shown in Figure 2a and b (N.B. *y*-axis scale is 10× smaller than in Figure 1, as tetraploidy is much lower in frequency than trisomy). No statistically significant increase in tetrasomy 7 and 8 was detected in either $CD34^+$ or $CD34^-$ cells with increasing doses of hydroquinone (for the four tests of trend by chromosome and cell type, $P > 0.3$; Figure 2a and b). Levels of tetrasomy 7 and their trends appeared quite similar to those in the corresponding cell types in tetrasomy 8. In fact, tetrasomy 7 and 8 frequently appeared to have occurred simultaneously in the same cells. Therefore, the majority of tetrasomic cells observed were most likely tetraploid. Hydroquinone treatment did not significantly increase the overall number of tetraploid cells at the concentrations used in our experiments.

Effect of hydroquinone on levels of monosomy

Cells with one hybridization signal were considered to be monosomic, although probe overlap may account for some apparent monosomy. Figure 3 shows the frequencies of monosomy 7 and 8 in the two different cell types following exposure to hydroquinone. Differences in the dose–response curves were observed between $CD34^+$ and $CD34^-$ cells. Significant dose-dependent increases in monosomy 7 were detected in both $CD34^+$ ($P_{\text{trend}} < 0.001$) and $CD34^-$ cells ($P_{\text{trend}} = 0.002$) (Figure 3a). A significant dose-dependent increase in monosomy 8 was found only in $CD34^+$ cells ($P_{\text{trend}} < 0.001$), and not in $CD34^-$ cells ($P_{\text{trend}} > 0.2$) (Figure 3b). The magnitude of the increase in monosomy 7 and 8 in $CD34^+$ cells was similar at ~3-fold, which was much greater than that observed in $CD34^-$ cells. In these non-progenitor $CD34^-$ cells, significantly higher background levels of monosomy 7 and 8 were observed compared with those found in $CD34^+$ cells ($P < 0.001$). The reason for this higher background level of monosomy in $CD34^-$ cells is unclear, but is most likely artifactual as trisomy of chromosomes 7 and 8 was actually higher in untreated $CD34^+$ cells than in $CD34^-$ cells (Figure 1).

Discussion

Our laboratory and others have reported previously that the polyphenolic metabolites of benzene, including hydroquinone, cause various forms of genotoxicity in human peripheral blood lymphocytes and cultured human cell lines, including increased levels of micronuclei (24,25), sister chromatid exchanges (26), classical chromosome aberrations (27), aneusomy of specific chromosomes as measured by FISH (18,20,28), oxidative DNA damage (29) and formation of DNA adducts (30). Terminally differentiated peripheral lymphocytes, while useful as surrogates in biological monitoring or epidemiological studies, are not the target cells for the induction of leukemia by benzene or other chemicals. Leukemia is thought to arise as a result of genotoxic damage to pluripotent stem cells or early progenitor cells which carry the CD34 surface antigen ($CD34^+$ cells) (21). Adult human blood contains very few $CD34^+$ cells (~1 in 10^6 cells) and human bone marrow is difficult to obtain. Fortunately, human cord blood is particularly rich in $CD34^+$ cells. Here, we have developed a protocol for the treatment of human cord blood with a chemical agent, in this case the

benzene metabolite hydroquinone, for 48 h and the subsequent isolation of $CD34^+$ and $CD34^-$ cell populations. We have performed interphase cytogenetics on chromosomes 7 and 8, which are often altered numerically in human leukemias. We found that $CD34^+$ cells were highly susceptible to aneusomy induction by hydroquinone and that doses as low as 2 μM produced significant changes in trisomy of chromosomes 7 and 8. Generally, $CD34^+$ cells were much more susceptible than $CD34^-$ cells to aneusomy induction by hydroquinone.

The sensitivity of $CD34^+$ progenitor cells to hydroquinone is quite remarkable in comparison with that of lymphocytes and human cell lines. Typically, hydroquinone concentrations >50 μM are required to produce significant genetic damage in adult lymphocytes (24,28) and >25 μM in other cell lines (31). In contrast, 2–3-fold increases in monosomy and trisomy of chromosomes 7 and 8 were observed in the present study in $CD34^+$ cells treated with 2 or 10 μM hydroquinone compared with control cells. We also attempted to apply FISH in granulocytes isolated from red blood cells in the current study in order to compare their sensitivity to the aneugenic effects of hydroquinone. We found it difficult to accurately score signals in the multilobed nuclei of granulocytes. The limited data obtained showed that granulocytes were less sensitive than $CD34^+$ cells to aneusomy induction by hydroquinone, but were more susceptible than $CD34^-$ cells. Significant dose–responses in granulocytes were observed for trisomy 7 ($P_{trend} < 0.01$) and 8 ($P_{trend} < 0.05$), but not for monosomy 7 and 8. A large proportion of the granulocytes examined were probably differentiated from $CD34^+$ cells because the original granulocytes are unlikely to survive 3 days in culture. Thus, the intermediate sensitivity of the granulocytes studied here could reflect their origin from two sources, the original blood sample and differentiated $CD34^+$ cells.

The finding that $CD34^+$ progenitor cells are more sensitive to the genotoxic effects of hydroquinone than $CD34^-$ cells, lymphocytes, granulocytes and cell lines is somewhat surprising. One might have expected these early progenitor cells to be more resistant to genetic damage than other terminally differentiated cells so that they were preserved in the living organism. However, the results described here clearly show that this is not the case, at least *in vitro*. Our data are moreover, consistent with other recent studies of the effect of benzene administration in the mouse, where $CD34^+$ cells in bone marrow also showed higher levels of aneusomy induction than $CD34^-$ cells (32).

The reason for the greater sensitivity of $CD34^+$ cells to hydroquinone is unclear, but one should bear in mind that the conditions used in the *in vitro* experiments described here are somewhat artificial, in that the culture environment strongly favors the growth and survival of $CD34^+$ cells. One possible explanation of our data is that, under these conditions, the $CD34^+$ cells divide faster than other cells in the culture and, therefore, express greater levels of aneusomy because they are more likely to exhibit treatment-related abnormalities in chromosome segregation as they go through mitosis. We are currently investigating this hypothesis in additional experiments. However, we expect that the 'faster growth' explanation cannot fully explain the high sensitivity of $CD34^+$ cells because adult and cord blood isolated lymphocyte cultures, provided with mitogens and growth factors to grow rapidly, are still much less susceptible to hydroquinone treatment than $CD34^+$ cells isolated from treated cord blood.

Another possible explanation may be that hydroquinone is converted to the highly toxic 1,4-benzoquinone, a potent spindle poison, and free radicals by myeloperoxidase, which is present in $CD34^+$ cells (5) but not in lymphocytes. However, HL60 cells also contain high levels of myeloperoxidase and they are several-fold less sensitive than $CD34^+$ cells to the aneusomy-inducing effects of hydroquinone. Thus, other explanations are likely and should be the subject of future research. Interestingly, levels of monosomy 7 were higher than monosomy 8 in $CD34^+$ cells and, conversely, levels of trisomy 8 were higher than trisomy 7. Monosomy 7 and trisomy 8 are common clonal numerical chromosome aberrations found in acute myeloid leukemias that arise in the stem-cell compartment (33) and are associated with chemical exposure and cigarette smoking (11,12,15). Our finding that the benzene metabolite hydroquinone is a potent inducer of monosomy 7 and trisomy 8 in $CD34^+$ stem or progenitor cells further implicates this metabolite in benzene-induced leukemogenesis. However, as discussed above, the ultimate metabolite responsible for the aneusomy-inducing effects of hydroquinone is most likely its oxidation product 1,4-benzoquinone (34).

The studies described here used $CD34^+$ progenitor cells from human cord blood, an excellent source of these cells. While the actual target for benzene metabolites in leukemia induction would be $CD34^+$ cells in bone marrow, which contains high levels of $CD34^+$ cells, normal bone marrow samples are more difficult to obtain, and the volume of recoverable bone marrow cells is much smaller. As a result, the total amount of $CD34^+$ cells obtainable is lower from bone marrow than from cord blood. We have been able to measure background aneusomy levels by FISH in $CD34^+$ and $CD34^-$ cells from one bone marrow sample. Preliminary results (data not shown) show that the frequency of trisomy 8 was higher in $CD34^+$ than in $CD34^-$ cells similar to those found in untreated cord blood. Studies are underway to determine the background rates and effects of hydroquinone on aneusomy in $CD34^+$ cells from bone marrow.

Under the methodology described in the present study, we have exposed whole cord blood to hydroquinone in culture and then subfractionated the cells after 72 h. An alternative experimental protocol would have been to isolate $CD34^+$ cells, expose them to hydroquinone and then culture them. Indeed, this was our initial approach, but we found that relatively few cells survived this protocol making scoring and statistical analysis difficult. Thus, we established an approach where $CD34^+$ cells would flourish in a growth factor-rich, whole-blood environment resulting in high yields. This cell culture protocol should be suitable for testing other candidate leukemogens for their ability to induce specific aneusomies and other types of leukemia-related effects in $CD34^+$ early progenitor cells.

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