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Characterization of Micronuclei Induced in Human Lymphocytes by Benzene Metabolites[1]

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ABSTRACT

Benzene is an established human leukemogen. Workers occupationally exposed to benzene exhibit increased frequencies of both structural and numerical chromosomal aberrations in their peripheral blood lymphocytes. The metabolite(s) responsible for these chromosomal aberrations has not yet been identified. Using a modified micronucleus assay, we have examined the ability of the metabolites of benzene to induce chromosomal damage in human lymphocytes. An antikinetochore antibody was used to distinguish micronuclei that have a high probability of containing a whole chromosome (kinetochore positive) from those containing acentric fragments (kinetochore negative). *In vitro* treatments with the benzene metabolites hydroquinone, 1,4-benzoquinone, phenol, and catechol resulted in significant increases in micronuclei formation. Phenol, catechol, and 1,4-benzoquinone treatments resulted in moderate (2- to 5-fold) increases in micronuclei, whereas hydroquinone treatments resulted in a larger (11-fold) increase in micronuclei. Significant dose-related increases in kinetochore-positive micronucleated cells were not observed following 1,4-benzoquinone treatment but were observed following treatment with phenol, catechol, and hydroquinone. The higher efficacy of hydroquinone in inducing both total micronuclei and kinetochore-positive micronucleated cells when compared with catechol, phenol, and 1,4-benzoquinone suggests that hydroquinone is a major contributor to the clastogenicity and aneuploidy observed in the lymphocytes of benzene-exposed workers. Other metabolites may also contribute, however, to the genotoxic effects of benzene. Since consistent chromosomal aberrations are often observed in human leukemias, the ability of the phenolic metabolites of benzene to induce chromosomal damage in human cells also implicates them in benzene-induced leukemia.

INTRODUCTION

Occupational exposure to benzene has been associated with aplastic anemia, leukemia, and other related blood disorders (1, 2). Benzene itself is unlikely to be the actual toxicant but rather is converted to bioactive metabolites which cause toxicity to the bone marrow (3, 4). Recent studies from our laboratory (5, 6) have shown that myelotoxicity similar to that observed following exposure to benzene can be produced by the coadministration of PH[3] and HQ and have implicated BQ as the actual toxicant. These results have added considerable support to the research of previous investigators which has implicated the phenolic and quinonoid metabolites of benzene as the metabolites that are most likely responsible for toxicity (7-10).

Due to the close association between myelotoxicity and the subsequent development of leukemia (11-13), it is possible that the same metabolites that are responsible for myelotoxicity are also responsible for leukemogenesis. The fundamental mechanisms underlying benzene-induced leukemia are still unknown. However, extensive *in vitro* genotoxicity testing has indicated that benzene is weakly mutagenic or nonmutagenic in standard mutation assays but does cause chromosomal aberrations (2, 14, 15). In addition, benzene has been reported to interfere with normal mitosis and to result in aneuploid daughter cells. *In vivo* genotoxicity studies have also demonstrated that benzene causes chromosomal damage that results in dramatic increases in structural chromosomal aberrations and micronucleated erythrocytes (2, 14, 15).

Cytogenetic studies in benzene-exposed workers have reported a similar profile of genotoxicity. Increased frequencies of structural chromosomal aberrations in the lymphocytes of benzene-exposed workers have been reported by numerous investigators (16, 17). Elevated frequencies of aneuploid cells have also been observed in the lymphocytes of workers with occupational exposure to benzene (18-20). Based on these observations, we decided to investigate the ability of the principal phenolic and quinonoid metabolites of benzene to induce structural and numerical chromosomal aberrations by utilizing a cytokinesis-block micronucleus assay in treated human peripheral lymphocytes (21). This method utilizes addition of cytochalasin B to cultures in order to block cells in cytokinesis. Such treatment results in multinucleate cell formation. Only binucleated interphase cells (or those that have undergone one mitosis) are identified and scored for the presence of micronuclei. This method has been shown to increase the ability to detect significant increases in the induction of micronuclei (22). Since micronuclei can be formed from entire chromosomes and/or chromosome fragments, the induction of micronuclei in lymphocytes by the metabolites of benzene provides a measure of genotoxicity which may be similar to that seen *in vivo*. In addition, we have used an antikinetochore antibody technique (23) to distinguish micronuclei that have a high probability of containing entire chromosomes from micronuclei containing only chromosome fragments in order to identify the relative potential for induction of aneuploidy and clastogenicity of the various metabolites.

MATERIALS AND METHODS

Culture and Treatment Conditions. Heparinized whole blood (35-70 ml) samples were drawn by venipuncture from the same healthy adult male for all experiments to eliminate donor-to-donor variability. Lymphocytes were isolated on Ficoll-Paque (Pharmacia, Piscataway, NJ) density gradients and were cultured at 37°C for 72 h in a 5% CO_2 atmosphere at an initial density of 0.5×10^6 cells/ml. Culture medium consisted of RPMI 1640 supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin (all from Gibco, Grand Island, NY), 10% fetal bovine serum (HyClone, Logan, UT), and 1.5% phytohemagglutinin (HA 15, Burroughs-Wellcome, Greenville, NC). Cytochalasin B (Sigma, St. Louis, MO) (3 μg/ml final concentration) was added at 44 h incubation and cells were harvested onto slides at 72 h as previously described (22).

For treatments, chemicals were solubilized in phosphate-buffered

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[3] The abreviations used are: PH, phenol; HQ, hydroquinone; BQ, 1,4-benzoquinone; CT, catechol; DMSO, dimethyl sulfoxide; DAPI, 4',6-diamidino-2-phenylindole.

saline and added in a total volume of 15 μl/ml of culture at 24 h after culture initiation; cells were harvested at 72 h. In experiments utilizing BQ, cells were washed at 41–43 h (late G_2, early M_1) and pulse treated for 10–30 min with appropriate concentrations of BQ in phosphate-buffered saline. This was necessary to prevent the reaction of BQ with proteins and sulfhydryl-containing compounds in the media. Cells were then resuspended in complete medium and cultured until harvest at 72 h. BQ stock solutions were prepared in DMSO (final concentration of DMSO in culture was 0.25%). Treatment with colchicine, a positive control, was performed from 24–44 h. Cells were then washed to release from block and were resuspended in complete medium for the remainder of the culture period.

Chemicals. Phenol (99+%), catechol (99+%), hydroquinone (99+%), and 1,4-benzoquinone (98+%), were purchased from Aldrich, Milwaukee, WI. Colchicine (96%) was purchased from Sigma. DMSO (reagent grade) was purchased from J. T. Baker, Phillipsburg, NJ.

Standard Micronucleus Assay. For micronucleus analysis, slides were fixed immediately in absolute methanol, dried, and stained with May-Grunewald Giemsa according to the method of Fenech and Morley (21). Slides were randomized and coded; a minimum of 1000 binucleated cells per point were scored when possible (500/duplicate culture) for micronuclei; 400 cells/point were scored (200/duplicate) for replicative indices according to established criteria (22). Cell viability was determined at harvest on all cultures by trypan blue dye exclusion.

Antikinetochore Assay. Detailed procedures for performing the antikinetochore assay have been described previously (23). Briefly, following fixation of cells in methanol, an antikinetochore antibody is applied in 0.1% Tween 20. A fluoresceinated rabbit anti-human antibody is then applied, and the nucleus is stained with DAPI. Slides are scored for micronuclei by using simultaneous phase contrast and DAPI fluorescence. After a micronucleated cell is located, the presence of a kinetochore in the micronucleus is determined by using fluorescein filter settings. This analysis was performed in parallel with the standard micronucleus assay described above; the number of micronucleated cells and those containing kinetochores were scored in 1000 binucleated cells/point.

Statistical Analyses. In order to examine the dose-response relationships, a trend test based on binomial data utilizing a $2 \times K$ contingency table (24), was performed on the total number of micronucleated cells and on the kinetochore-positive micronucleated cells for each of the tested metabolites. Further statistical analyses to compare each of the treatments with the controls were performed, using a 1-tailed Fisher exact test. Due to the large number of comparisons performed, Bonferroni's correction for multiple comparisons was used to confine the overall probability of Type I error for each chemical to 0.05. Statistical analyses for kinetochore-positive micronucleated cells were performed only for treatments in which a statistically significant increase in micronucleated cells was observed. Analysis of variance showed that control values did not differ significantly from experiment to experiment, therefore data for the various experiments were pooled for statistical analysis. The distribution of micronuclei per binucleated cell was compared with expected values based on a Poisson distribution using a χ^2 goodness of fit analysis.

RESULTS

All of the tested benzene metabolites induced significant dose-related increases in micronuclei in cultured human lymphocytes. A summary of the micronuclei data pooled from both the standard and the antikinetochore-modified micronucleus assays for the various benzene metabolites is shown in Table 1. The spindle disrupting agent colchicine was used as a positive control.

BQ treatment resulted in a weak (approximately 2-fold), but statistically significant, increase in micronuclei at 2.5 and 5 μM (Table 1). A weak dose response was also observed over this concentration range. Increased frequencies of micronuclei were not observed at higher concentrations of BQ (10 to 80 μM), although high toxicity did not permit the analysis of a large number of cells. Acutely toxic effects were observed at concentrations as low as 1.25 μM (Table 2), and these did not seem to correlate with the increase in micronuclei. Interestingly, cells that survived BQ exposure for 72 h showed essentially no decrease in replicative index (Table 2), indicating that cell death occurred early in the culture period.

The treatment of lymphocytes with PH resulted in a significant dose-related increase in micronuclei at concentrations ranging from 250 to 750 μM (Table 1). An approximately 3- to 4-fold increase in micronuclei was observed at 750 μM or greater concentrations. Cell proliferation, as estimated by the replicative index, and cell killing, as estimated by percentage of cells viable at harvest, were not greatly affected over the concentration range tested (Table 2).

All tested concentrations of CT showed an increase in micronuclei (Table 1). Although minor 2- to 3-fold increases in micronuclei were observed at CT concentrations up to 100 μM, a large increase (4- to 5-fold) in micronuclei was observed at 200 μM. This increase in micronuclei formation was accompanied by decreases in cell viability and in the replicative index (Table 2).

Overall, HQ was the most effective inducer of micronuclei with an 11-fold increase at 125 μM and a clear dose response from 25 to 125 μM (Table 1). The dose-related increase in micronuclei formation was paralleled by a striking reduction in the replicative index and cell viability (Table 2; Fig. 1).

The distribution of micronuclei per binucleated cell for each agent and dose is shown in Table 1. Twenty-nine of the 36 distributions differed significantly from the Poisson due to an excess of cells with multiple micronuclei. This excess indicates that the number of micronuclei per cell is not random; *i.e.*, a cell with one micronucleus is more likely to contain additional micronuclei.

A modified micronucleus assay using an antikinetochore antibody was used to distinguish micronucleated cells in which the micronucleus contains a kinetochore, and therefore probably an entire chromosome (kinetochore positive), from those in which the micronucleus contains a chromosomal fragment (kinetochore negative). A photograph of a micronucleated cytokinesis-blocked lymphocyte is shown in Fig. 2. Fig. 2*A* shows an HQ-treated binucleated lymphocyte containing a micronucleus by using the phase/DAPI filter setting. The same cell is shown in Fig. 2*B*, using a fluorescein filter setting. Numerous kinetochores are visible in the main nuclei and 3 kinetochores are visible in the micronucleus. This kinetochore-positive micronucleated cell represents a cell with a high potential for aneuploidy if cytokinesis had been allowed to occur. The induction of kinetochore-positive and -negative micronucleated cells following treatment with the benzene metabolites is shown in Table 3. The assay assesses the number of micronucleated cells rather than total number of micronuclei per cell, since the induction of only one kinetochore-positive micronucleus results in a cell with an increased potential for aneuploidy (23).

Statistically significant increases in micronucleated cells were observed for the three phenolic metabolites, PH, CT, and HQ (Table 3). A significant increase was not observed for BQ, however, probably due to toxicity and the resulting reduction in sample size. Significant dose-related increases in kinetochore-positive micronucleated cells were also observed for HQ, PH, and CT, suggesting that each of these chemicals are likely aneuploidy-inducing agents in human lymphocytes.

DISCUSSION

The observation of chromosomal aberrations in benzene-exposed workers, combined with the apparent lack of mutagen-

Table 1 *Induction and distribution of micronuclei in cytokinesis-blocked human lymphocytes following treatment with benzene metabolites*

Chemical	Concentration (μM)	Total MN[a]	Total MN cells	Total BN cells scored	Standardized per 1000 BN		No. of cells with indicated no. of micronuclei				
					MN	MN cells	1	2	3	4	≥5
Controls											
PBS		45	37	7,800	6	5	33	1	2	1	0
DMSO (0.25%)		64	50	8,000	8	6	43	4	1	1	1
Colchicine[b,c]	0.025	10	5	1,000	10	5	3	1	0	0	1
	0.050[d]	61	39	1,000	61	39	25	9	3	1	1
	0.075[d]	106	50	1,000	106	50	28	9	2	6	5
	0.100[d]	177	81	1,241	143	65	41	16	11	5	8
1,4-Benzoquinone[c]	0.25	5	4	1,000	5	4	3	1	0	0	0
	0.63	6	6	2,000	3	3	6	0	0	0	0
	1.25	22	18	4,000	6	5	16	1	0	1	0
	2.50[d]	147	83	10,735	14	8	67	6	1	2	7
	3.75	28	12	2,000	14	6	9	0	0	1	2
	5[d]	79	60	6,000	13	10	48	9	1	0	2
	10	7	3	591[e]	12	5	1	1	0	1	0
Phenol[c]	50	15	14	2,000	8	7	13	1	0	0	0
	250[d]	25	23	2,000	13	12	21	2	0	0	0
	500[d]	16	15	1,000	16	15	14	1	0	0	0
	750[d]	24	19	1,000	24	19	16	2	0	1	0
	1,000[d]	18	16	1,000	18	16	15	0	1	0	0
	2,000[d]	22	20	1,000	22	20	18	2	0	0	0
	5,000[d]	16	16	1,000	16	16	16	0	0	0	0
Catechol[c]	0.5[d]	14	12	1,000	14	12	10	2	0	0	0
	5.0[d]	14	12	1,000	14	12	10	2	0	0	0
	50[d]	15	14	1,000	15	14	13	1	0	0	0
	100[d]	16	16	1,000	16	16	16	0	0	0	0
	200[d]	28	25	1,000	28	25	23	1	1	0	0
	250	4	4	290[f]	14	14	4	0	0	0	0
Hydroquinone[c]	2.0	3	3	1,000	3	3	3	0	0	0	0
	6.0	1	1	1,000	1	1	1	0	0	0	0
	12	1	1	1,000	1	1	1	0	0	0	0
	15	5	4	1,000	5	4	3	1	0	0	0
	25	21	17	2,000	11	9	14	2	1	0	0
	50	11	10	1,000	11	10	9	1	0	0	0
	75[d]	42	37	2,000	21	19	33	3	1	0	0
	100[d]	118	88	2,244	53	39	71	10	4	2	1
	125[d]	123	100	2,000	62	50	88	6	3	2	1
	150[d]	108	75	1,778	61	42	57	13	3	0	2

[a] MN, total number of micronuclei observed in binucleated cells; MN cells, total number of micronucleated cells observed; BN, number of binucleated cells; PBS, phosphate-buffered saline.
[b] Positive control.
[c] Slope of the dose response for the number of micronucleated cells induced by treatment was significantly different from expected using a trend test based on binomial data. χ^2 *P* values were all $\ll 0.001$.
[d] Significantly different from control using a 1-tailed Fisher exact test. Critical *P* values for each chemical were determined by using Bonferroni's correction for multiple comparisons and were as follows: colchicine, 0.013; 1,4-benzoquinone, 0.0071; phenol, 0.0071; catechol, 0.0083; hydroquinone, 0.0046.
[e] Only 591 cells scored due to toxicity.
[f] Only 290 cells scored due to toxicity.

icity of benzene, indicates that these aberrations may be important in benzene-induced leukemia. The micronucleus assay is a simple and rapid technique which permits the identification of agents which cause both structural and numerical aberrations. We have, therefore, used this approach in order to study the potential role of various benzene metabolites in benzene-induced leukemia.

The use of the cytokinesis-blocked micronucleus assay and the antikinetochore modification of this assay indicated that all of the tested phenolic and quinonoid metabolites of benzene were genotoxic. BQ, PH, and CT treatments resulted in minor to moderate (approximately 2- to 5-fold) increases in micronuclei, whereas HQ treatments resulted in a larger (11-fold) increase in micronuclei. Significant dose-related increases in kinetochore-positive micronucleated cells were not observed for BQ. The dose-related increases observed with PH and CT were relatively minor and were largely due to the increases observed at the 5000 μM concentration of PH and the 200 μM concentration of CT, respectively. The treatment of cells with HQ resulted in significant increases in kinetochore-positive cells at 75 μM or greater concentrations.

The higher potency of HQ in inducing both total micronuclei and kinetochore-positive micronucleated cells when compared with CT and PH suggests that HQ may be the most genotoxic of these phenolic metabolites *in vitro*. It is, therefore, likely to be a major contributor to the clastogenicity and aneuploidy observed in the lymphocytes of benzene-exposed workers. CT and PH, however, could also conceivably contribute to the structural and numerical aberrations seen in those workers.

The nature of the actual chemical species involved in the induction of micronuclei by HQ is uncertain. It seems likely that these effects are mediated by its oxidation products, the 1,4-benzosemiquinone radical and/or 1,4-BQ. Extensive efforts on our part, however, failed to demonstrate qualitatively large (greater than 2-fold) increases in micronuclei in human lymphocytes following BQ exposure. Experiments utilizing dose fractionation treatment protocols aimed at delivering this highly reactive and toxic compound to the cells over a longer period of time were also unsuccessful. Attempts to treat the cells with BQ at different stages of the cell cycle also failed to yield a higher induction of micronuclei formation. Although these experiments appear to indicate that either HQ itself or the semiquinone radical rather than BQ was actually responsible for the genotoxicity of HQ, the intracellular conversion of

Table 2 *Effect of in vitro treatment with phenolic and quinonoid metabolites of benzene on cell cycle kinetics and cell viability*

Chemical	Concentration (μM)	% mononuclear[a]	% binuclear[a]	% > binuclear[a]	RI[b]	% of viable cells[c]
Controls						
PBS		19	58	23	2.04	91
DMSO (0.25%)		19	56	25	2.06	88
Colchicine[d]	0.025	28	61	11	1.83	80
	0.050	55	40	5	1.50	68
	0.075	76	22	2	1.26	74
	0.100	82	15	3	1.21	64
1,4-Benzoquinone	0.63	26	59	15	1.90	86
	1.25	34	51	15	1.81	56
	2.5	34	54	12	1.78	48
	3.75	24	48	28	2.05	46
	5	25	55	20	1.96	30
	10	26	52	22	1.96	36
	20	16	62	22	2.06	34
	40	17	62	21	2.04	32
	60	15	66	19	2.03	27
	80	22	56	22	2.00	21
Phenol	50	23	49	28	2.05	98
	250	23	61	16	1.93	97
	500	28	63	9	1.81	95
	750	23	68	9	1.86	98
	1000	38	54	8	1.70	89
	2000	30	66	4	1.74	87
	5000	82	18	0	1.18	74
Catechol	0.5	23	53	24	2.02	95
	5	23	55	23	2.00	96
	50	36	61	3	1.68	91
	100	68	32	0	1.32	85
	200	90	10	0	1.10	66
	250	89	11	0	1.11	61
Hydroquinone	2	11	65	24	2.14	89
	6	27	66	7	1.79	94
	12	29	66	5	1.76	88
	15	20	61	19	1.45	94
	25	45	53	2	1.57	76
	50	60	39	1	1.43	35
	75	40	54	6	1.66	68
	100	61	34	5	1.43	59
	125	68	31	1	1.33	54
	150	91	8	1	1.10	42

[a] Percentage of cells containing 1, 2, and 3 or more nuclei; 400 cells were scored for each concentration.
[b] RI, replicative index:

$$RI = \frac{(\%\text{ of mononucleated cells}) + (2 \times \%\text{ of binucleated cells}) + (3 \times \%\text{ of} > \text{binucleated cells})}{100}$$

[c] Viability was determined at harvest (72 h) by scoring 200 cells/point for trypan blue dye exclusion.
[d] Positive control.

HQ to BQ may be critical. Due to its high reactivity, the extracellular addition of BQ may deplete reduced glutathione and critical membrane thiols, resulting in toxicity without reaching the DNA and exerting a genotoxic effect. HQ, on the other hand, could enter cells and generate BQ or the semiquinone radical close to target sites within the cells. Recent studies in our laboratory with ascorbic acid support this notion, since inclusion of this reducing agent in the incubation medium inhibits both the genotoxic and toxic effects of hydroquinone. This indicates that an oxidation product of HQ is most likely responsible for both toxicity and genotoxicity.[4]

The cytotoxic effects of BQ were observed at considerably lower concentrations (1.25 μM) than that seen with PH, CT, or HQ. Further, the measures of toxicity and replication reported in this paper are likely to underestimate the actual toxicity occurring in culture since only cells with relatively intact cell membranes or nuclei at 72 h can be detected by the trypan blue exclusion assay. At high concentrations of the tested agents,

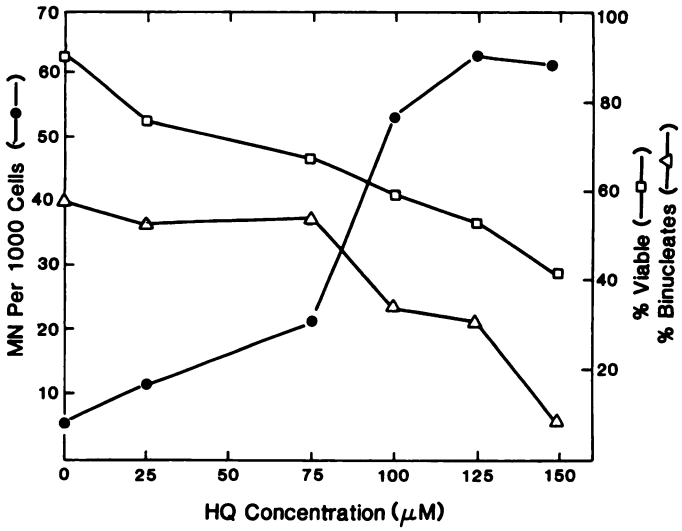

Fig. 1. Total number of micronuclei per 1000 binucleated cells (●), percentage of cells viable at 72 h harvest (□), and percentage of cells that are binucleated (△) after treatment with 25 to 150 μM HQ.

[4] M. Robertson and M. T. Smith, unpublished results.

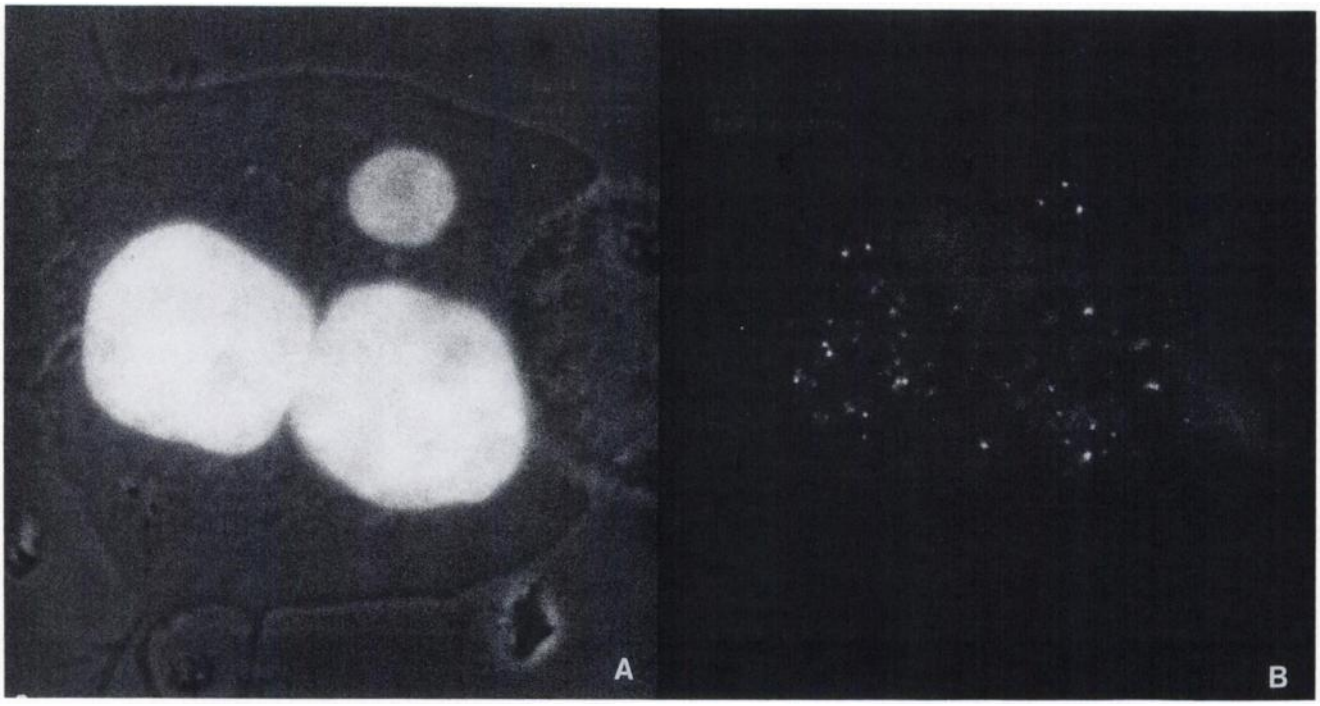

Fig. 2. *A*, a hydroquinone-treated binucleated lymphocyte containing a micronucleus, using the phase/DAPI filter setting so that the cell membrane and nuclei can be seen. *B*, the same cell, using the fluorescein filter setting. Numerous kinetochores are visible in the main nuclei and 3 kinetochores are visible in the micronucleus.

cells which had apparently died early in cell culture were observed in the micronucleus slide preparations, but were probably lost to assay by the trypan blue exclusion technique at 72 h. Interestingly with BQ and HQ treatments, these cells with small condensed nuclei exhibited a bright yellow fluorescence when excited with blue light (Fig. 2*B*). This suggests that a DNA or protein adduct with BQ may act as a fluorochrome. Previous cytogenetic studies with human lymphocytes (25) have reported significant toxicity only after relatively high concentrations (300 μM) of BQ were reached. The difference in effective concentration between their study and our own is probably related to the composition of the media in which the cells were treated. Erexson *et al.* (25) treated their cells with BQ in complete medium, whereas in our experiments the lymphocytes were washed and resuspended in phosphate-buffered saline prior to BQ exposure. Since BQ is likely to bind to proteins or to be reduced or conjugated by thiols (26), considerably more BQ would need to be added in complete medium in order to reach an effective concentration. During preliminary experiments with BQ in our laboratory, the treatment of lymphocytes in complete medium produced variable results and much higher concentrations of BQ were required.

Recently, a number of genotoxicity studies demonstrating the induction of aberrations or micronuclei by the various metabolites of benzene have been reported (27–30). Generally, the results of these studies are in agreement with our findings that HQ is a potent inducer of micronuclei formation. Using a single p.o. dose of PH, CT, and HQ, Gad-El Karim *et al.* (27) reported that only HQ administration induced a significant increase in micronuclei in the polychromatic erythrocytes of treated mice. Similar results were reproted by Cirrani *et al.* (29), in that, of a variety of benzene metabolites tested for inducing micronuclei in the bone marrow of pregnant mice and in the liver of the fetuses, HQ was the most potent inducer of micronuclei in the fetal liver. HQ also showed modest increases in micronuclei in the bone marrow. All other metabolites showed modest or negative genotoxic effects. Shimada *et al.* (28) reported that, in addition to HQ, PH was able to induce micronuclei in mouse bone marrow polychromatic erythrocytes *in vivo*. They also reported that HQ, PH, and CT were positive for inducing structural chromosomal aberrations in Chinese hamster ovary cells. BQ was inactive in both assays.

Crebelli *et al.* (30) reported that HQ was a potent inducer of mitotic segregants in *Aspergillus nidulans*. Positive effects were also observed at higher concentrations for CT and PH. The authors concluded that structural chromosomal aberrations were the primary effects caused by these phenolic metabolites and that the numerical abnormalities were secondary effects which appeared as a consequence of structural chromosomal damages. In contrast, our data in human cells indicate that HQ-induced numerical aberrations are a primary effect occurring concurrently with structural aberrations at relatively low concentrations of HQ.

The mechanism by which benzene and its metabolites cause aneuploidy is likely to be through interference with normal spindle formation and function. The potent inhibition of microtubule assembly and lymphocyte function by BQ and HQ metabolites has previously been demonstrated (9, 31). The mechanism by which HQ induces structural chromosomal aberrations is not as apparent, however, but may be related to its ability to produce oxygen radicals (8, 32) or through covalent binding to DNA and adduct formation. Clastogenicity could also result, however, from inhibition of DNA and RNA polymerases (33, 34) or other enzymes involved in DNA replication or transcription such as ligases or topoisomerases.

The observation that the metabolites of benzene are able to induce both structural and numerical aberrations provides insights into potential mechanisms underlying benzene-induced leukemia. A growing body of molecular and cytogenetic evidence indicates that chromosomal aberrations play an important role in the neoplastic development of certain tumors (35–37). The recent chromosomal localization of various oncogenes,

Table 3 *Induction of kinetochore-positive and -negative micronucleated lymphocytes following in vitro treatment with benzene metabolites*

Chemical	Concentration (μM)	Total cells scored	Micronucleated cells				Micronuclei			
			Total[a]	+[b]	−[c]	?[d]	Total[e]	+	−	?
Controls										
PBS		3000	16	8	7	1	19	10	8	1
DMSO (0.25%)		2000	15	9	6	0	21	11	7	0
1,4-Benzoquinone	2.5	2500	26	11	10	4	31	11	12	7
	5.0	2000	25	9	13	3	33	9	21	3
Phenol[f]	50	1000	10	8	2	0	10	8	2	0
	250	1000	14	3	9	2	15	3	10	2
	500	1000	15[g]	6	7	2	16	6	8	2
	750	1000	19[g]	7	9	3	24	11	9	4
	1000	1000	16[g]	7	9	0	18	9	9	0
	2000	1000	20[g]	5	14	1	22	7	14	1
	5000	1000	16[g]	14[g]	2	0	16	14	2	0
Catechol[f]	0.5	1000	12	5	7	0	14	6	8	0
	5.0	1000	12	4	8	0	14	5	9	0
	50	1000	14[g]	8	6	0	15	8	7	0
	100	1000	16[g]	7	9	0	16	7	9	0
	200	1000	25[g]	11[g]	14	0	28	14	14	0
	250	290[h]	4	3	1	0	4	3	1	0
Hydroquinone[f]	75	1000	21[g]	15[g]	5	1	23	16	6	1
	100	1000	43[g]	22[g]	17	4	54	26	18	10
	125	1000	61[g]	21[g]	39	1	76	27	48	1
	150	776[i]	39[g]	17[g]	9	13	56	18	23	15

[a] Number of micronucleated cells. These data are a subset of the data presented in Table 1.

[b] +, number of micronucleated cells or micronuclei containing at least one kinetochore-positive micronucleus.

[c] −, number of micronucleated cells or micronuclei lacking a kinetochore-positive micronucleus.

[d] ?, number of micronucleated cells or micronuclei that were unscorable for kinetochores.

[e] Number of micronuclei in binucleated cells. These data are a subset of the data presented in Table 1.

[f] Slope of the dose response for the number of kinetochore-positive micronucleated cells induced by treatment was significantly different from expected, using a trend test based on binomial data. χ^2 *P* values were as follows: phenol, 0.0065; catechol, 0.0419; hydroquinone, ≪0.001.

[g] Significantly different from control, using a 1-tailed Fisher exact test. Critical *P* values for each chemical were determined by using Bonferroni's correction for multiple comparisons and were as follows: 1,4-benzoquinone, 0.025; phenol, 0.007; catechol, 0.0083; hydroquinone, 0.013.

[h] Only 290 cells scored due to toxicity.

[i] Only 776 cells scored due to toxicity.

tumor suppressor genes, and growth factor genes indicates potential molecular mechanisms by which the loss, gain, or translocation of chromosomes or chromosomal segments may be involved in carcinogenic processes (38–41). In particular, consistent chromosomal aberrations are often observed in human leukemias and lymphomas (36, 42, 43) and have yielded considerable information regarding the mechanisms involved in these neoplasms (44). Similar studies are currently under way within our laboratory to identify specific patterns of chromosomal aberrations induced by the metabolites of benzene, to quantify subsequent changes in gene expression, and to relate these changes to the karyotypic abnormalities observed in leukemia patients with prior benzene exposure.

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