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Nonrandom Aneuploidy of Chromosomes 1, 5, 6, 7, 8, 9, 11, 12, and 21 Induced by the Benzene Metabolites Hydroquinone and Benzenetriol

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The loss and gain of whole chromosomes (aneuploidy) is common in the development of leukemia and other cancers. In acute myeloid leukemia, the loss (monosomy) of chromosomes 5 and 7 and the gain (trisomy) of chromosome 8 are common clonal chromosomal abnormalities. Here, we have tested the hypothesis that metabolites of the human leukemogen benzene cause a higher rate of gain and loss among the chromosomes involved in leukemogenesis and, as such, are nonrandom and selective in their effects. Human peripheral blood was exposed to two metabolites of benzene, namely, hydroquinone (HQ) and benzenetriol (BT), and the ploidy status of nine different chromosomes (1, 5, 6, 7, 8, 9, 11, 12, and 21) was examined using fluorescence in situ hybridization of metaphase spreads. Poisson regression was used to provide interpretable incidence rate ratios and corresponding *P* values for all nine chromosomes. Statistically significant differences were found between the sensitivity of the nine chromosomes to gain or loss. Chromosomes 5 and 7 were highly sensitive to loss following HQ and BT exposure, whereas chromosomes 7, 8, and 21 were highly sensitive to gain in comparison to other chromosomes. Significant support for the *a priori* hypothesis that chromosomes 5 and 7 are more sensitive to loss induced by HQ and BT than the other seven chromosomes was also obtained. These data support the notion that benzene metabolites affect the ploidy status of specific chromosomes more than others and may initiate or promote leukemia induction through these specific effects. Environ. Mol. Mutagen. 45:388–396, 2005.

Key words: hydroquinone; 1,2,4-benzenetriol; chromosomal aberrations; aneuploidy; fluorescence in situ hybridization; human leukemia

INTRODUCTION

Genes are unequally distributed among the 22 nonsex chromosomes (autosomes) in humans and these autosomes vary greatly in size and DNA content [Mendelsohn et al., 1973; Bentley et al., 2001; Young, 2002]. For example, chromosome 1 is rich in active genes and is the largest chromosome, whereas chromosomes 21 and 22, which make up chromosome group G, are the smallest [Young, 2002]. The loss and gain of whole chromosomes (aneuploidy) is common in the development of leukemia and other cancers and leads to alterations in gene expression [Young, 2002]. For example, in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), the loss of chromosomes 5 and 7 (monosomy) and the gain of chromosome 8 (trisomy) are common clonal chromosomal abnormalities [Le Beau, 1992; Boultwood and Fidler, 1995; Grimwade et al., 1998; Brozek et al., 2003]. One mechanism by which these selective aneuploidies arise could be nonspecific damage to the DNA or spindle apparatus followed by selection of clones harboring monosomy 5 and 7 or trisomy 8 because they grow faster than surrounding cells. Under this scenario, the initial damage that leads to the loss or gain of the chromosome, whether spontaneous or caused by chemicals, radiation, or a virus, is random rather than selective to the specific chromosomes. Here, we have tested an alternate mechanism for the production of these specific aneuploidies, namely, that metabolites of the leukemogenic chemical, benzene, cause a higher rate of chromosome gain and loss among the chromosomes involved in leukemogenesis and, as such, are selective in their effects.

Occupational exposure to benzene induces both numerical (aneuploidy) and structural chromosome aberrations in

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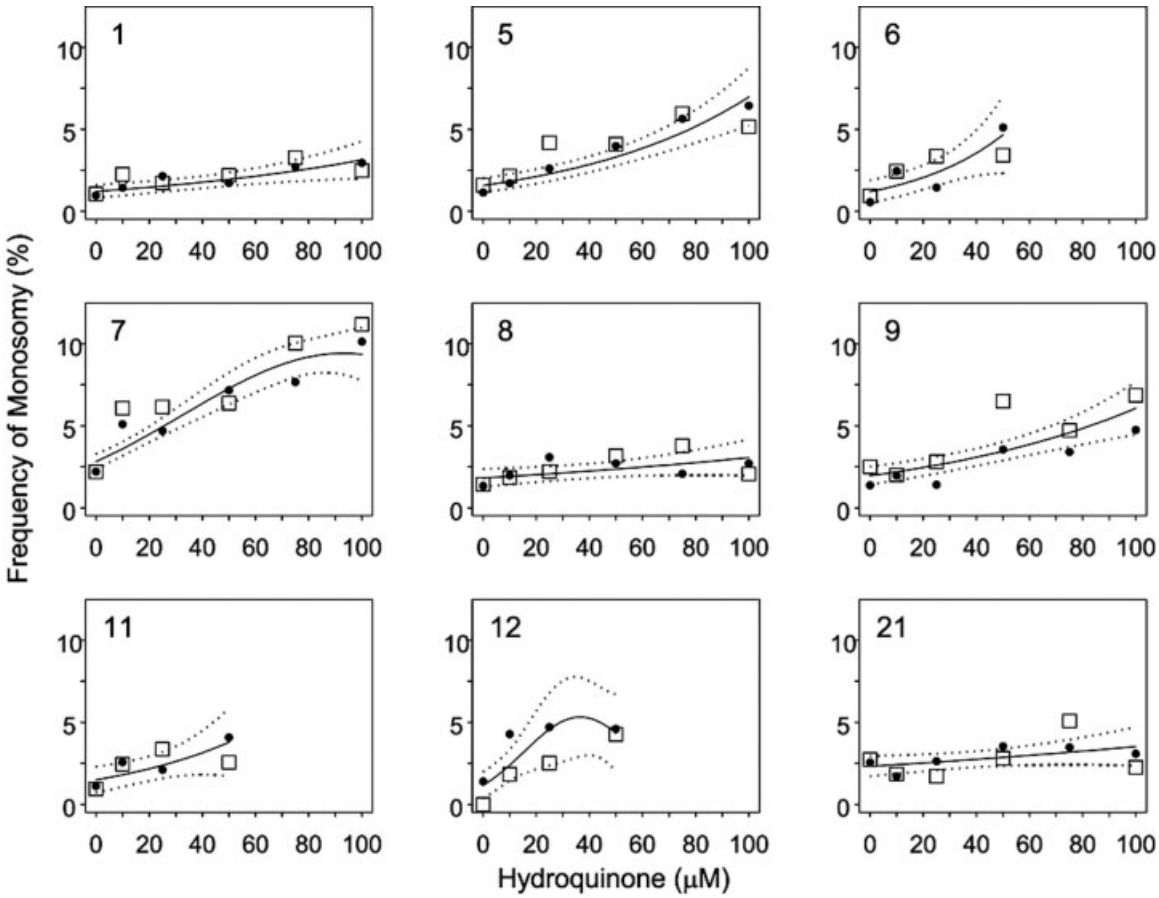

Fig. 1. Frequency of monosomy induced by HQ in chromosomes 1, 5, 6, 7, 8, 9, 11, 12, and 21. Mean values for monosomy of the nine chromosomes following treatment with 0, 10, 25, 50, 75, and 100 μM HQ in cultured human lymphocytes are presented for each blood donor (as open squares and filled circles). Each donor's blood was cultured in three separate experiments. The graphs represent the best log-linear (or log-quadratic) fit to the actual raw data (solid line) and the 95% pointwise confidence intervals as dashed lines. Data for chromosomes 1, 5, 6, 7, 8, 9, 11, 12, and 21 are shown sequentially with the chromosome number indicated in the top left corner. Only six chromosomes (1, 5, 7, 8, 9, and 21) were examined at higher doses of HQ (75 and 100 μM) due to its cytotoxicity such that fewer cells were obtained.

circulating blood lymphocytes [Tough et al., 1970; Erdogan and Aksoy, 1973; Forni, 1979; Whysner, 2000; Zhang et al., 2002a]. Early studies showed that the loss or gain of C-group chromosomes (6–12 and X) was often observed in benzene-associated leukemia patients [Forni and Moreo, 1967; Aksoy et al., 1974]. More recently, we have reported that the loss of chromosomes 5 and 7 (monosomy 5 and 7) and the gain of chromosomes 8 and 21 (trisomy 8 and 21) are significantly increased in benzene-exposed workers in comparison with controls [Smith et al., 1998; Zhang et al., 1998a]. In order to produce these chromosome-damaging effects, benzene must be metabolized to one or more genotoxic metabolites [Yager et al., 1990]. The most likely candidate toxic metabolites are 1,4-benzoquinone and 2-hydroxy-1,4-benzoquinone derived from the polyphenolic metabolites hydroquinone (HQ) and 1,2,4-benenetriol (BT), respectively [Smith et al., 1989; Zhang et al., 1993, 1994; Ross, 1996]. In previous studies, HQ and BT induced micronuclei in human lymphocytes [Yager et al., 1990; Robertson et al., 1991] and a myeloid cell line (HL60) [Zhang et al., 1993], and aneuploidy by disrupting microtubules [Zhang et al., 1994]. In addition, they induced the loss and long-arm deletion of chromosomes 5 and 7 in human lymphocytes [Zhang et al., 1998b]. HQ also increased the level of aneusomy of chromosomes 7 and 8 in human CD34-positive blood progenitor cells [Smith et al., 2000]. These results have been confirmed by other studies [Stillman et al., 1999, 2000; Chung and Kim, 2002]. In the present study, we have tested the ability of HQ and BT to produce selective chromosomal aneuploidy in human cells.

Previously, we reported that metabolites of 1,3-butadiene induced chromosome-specific aneuploidy (of chromosomes 12 and X but not 7 and 8) in treated human cells [Xi et al., 1997]. Here, we have tested the differential sensitivity of up to nine different chromosomes to the aneuploidy-inducing effects of HQ and BT. Specifically, we have examined the *a priori* hypothesis that chromosomes 5 and 7 are significantly more sensitive than other

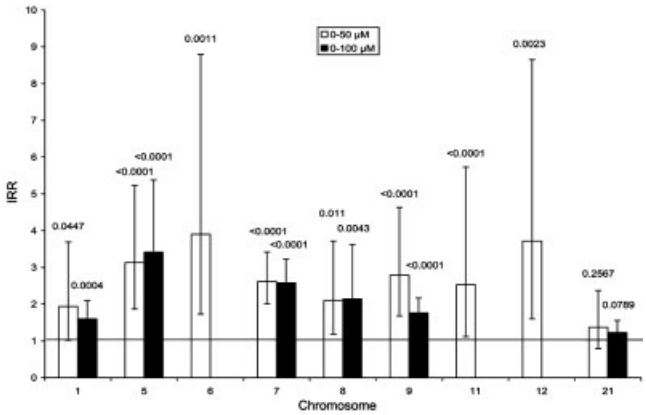

Fig. 2. Selective effect of HQ on monosomy of chromosomes 1, 5, 6, 7, 8, 9, 11, 12, and 21. The IRRs that describe how aneuploidy frequencies rise as HQ dose increases are shown for each chromosome tested over two dose ranges. Clear bars represent HQ treatments from 0 to 50 μM only, while shaded bars represent the HQ treatments that included the higher doses of 75 and 100 μM (for six chromosomes only). The error bars are shown as the 95% CIs and *P* values for dose-response are presented above them. IRRs, 95% CIs, and *P* values were calculated from Poisson regression models as described in text.

chromosomes to loss induced by these benzene metabolites because monosomy 5 and 7 are the most common cytogenetic abnormalities in chemotherapy-related [Pedersen-Bjergaard et al., 1995] and benzene-induced leukemia [Zhang et al., 2002a]. In order to do this, cultured human blood was treated with HQ and BT and numerical changes in up to nine chromosomes (1, 5, 6, 7, 8, 9, 11, 12, and 21) detected using fluorescence in situ hybridization (FISH). These chromosomes were chosen so as to include the largest and smallest chromosome groups (1 and 21) and most of the C-group chromosomes (6–12).

In order to compare the effects of benzene metabolites on different chromosomes, we have used Poisson regression to provide interpretable dose-response associations for HQ and BT and chromosome responses defined as incidence rate ratios (IRRs) and corresponding *P* values of the association for all nine chromosomes. We demonstrate the induction of selective aneuploidy in human cells by HQ and BT and show that chromosomes 5 and 7 are statistically more sensitive to monosomy induction in the presence of HQ and BT than other chromosomes. These data support the idea that benzene can initiate or promote leukemia induction by a nonrandom selective effect on the ploidy status of specific chromosomes.

MATERIALS AND METHODS

Whole Blood Cell Culture

Heparin-anticoagulated blood samples were obtained by venipuncture from two healthy female donors. Peripheral blood (0.4 ml) was cultured in 5 ml of medium consisting of RPMI-1640 supplemented with 2 mM L-glutamine, 10% fetal bovine serum (Hyclone, Logan, UT), 100 units/ml penicillin, 100 μg/ml streptomycin (all from Gibco, Grand Island, NY), and 1% phytohemagglutinin (HA15; Burroughs-Wellcome, Greenville, NC). All cultures were incubated at 37°C for 72 hr in a 5% CO_2 atmosphere. In order to obtain a sufficient number of metaphase spreads, colcemid (0.1 μg/ml) was added to each culture 4 hr before harvesting. The harvested cells were then placed in hypotonic solution (0.075 M KCl) for 25 min at 37°C and fixed three times with Carnoy's solution (methanol:glacial acetic acid = 3:1). The fixed cells were dropped onto glass slides, allowed to air-dry, and stored at −20°C in an N_2 atmosphere until use.

Treatment With Benzene Metabolites

HQ and BT (99%; both from Aldrich, Milwaukee, WI) were dissolved in phosphate-buffered saline (PBS; pH 7.4) immediately prior to treatment. At 24 hr after culture initiation, blood cells were treated with HQ (10, 25, 50, 75, and 100 μM) and BT (10, 25, and 50 μM) separately in the complete medium. All treatments were performed in duplicate for each dose and repeated twice. The treated cells were harvested after 48 hr of chemical exposure. The cytotoxicity of the chemicals was measured by evaluated cell viability. The Easy-Lyse whole blood erythrocyte lysing kit (Leinco Technologies, Ballwin, MO) was employed to lyse erythrocytes gently while maintaining the viability of unfixed lymphocytes. 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 with 1 ml washing buffer and centrifuged. The supernatant was decanted and the cells were checked for viability after staining with 0.16% Trypan blue diluted in PBS.

Fluorescence In Situ Hybridization

To determine the chromosomal alterations induced by HQ and BT in cultured human lymphocytes, we applied commercially available FISH probes to target a total of nine chromosomes in HQ-treated cells and six chromosomes in BT-treated cells. Fewer cells were obtained after BT treatment and so a lower number of FISH probes were applied. All probes were purchased from Oncor (Gaithersburg, MD) and hybridization was performed as suggested. The biotin-labeled probes were stained green (FITC) and the digoxigenin-labeled probes red (rhodamine). All nontargeted chromosomes were counterstained with a 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 detailed FISH procedure has been described previously [Zhang et al., 1998b]. Prior to scoring, the stained slides were randomized and coded. All of the well-spread and well-stained metaphase cells on each slide were scored. The criteria of scorable metaphase spreads were described previously [Smith et al., 1998; Zhang et al., 1998a]. Note that this type of analysis produces higher background frequencies of aneuploidy than does conventional analysis [Zhang et al., 1999]. The total number of scored metaphase cells in this study was 16,155 and the number of scored cells per dose by each FISH assay was greater than 300.

Statistical Analysis and Modeling

Poisson regression was applied in this study because it is commonly applied when the outcome variable is a count; because it can naturally adjust for differences in the denominator (total number of cells tested); and because it provides interpretable associations between two measures, called incidence rate ratios [McCullagh and Nelder, 1989], that describe how the aneuploidy frequency increases as the HQ/BT dose increases. For all models, we also tested for overdispersion by comparing the rela-

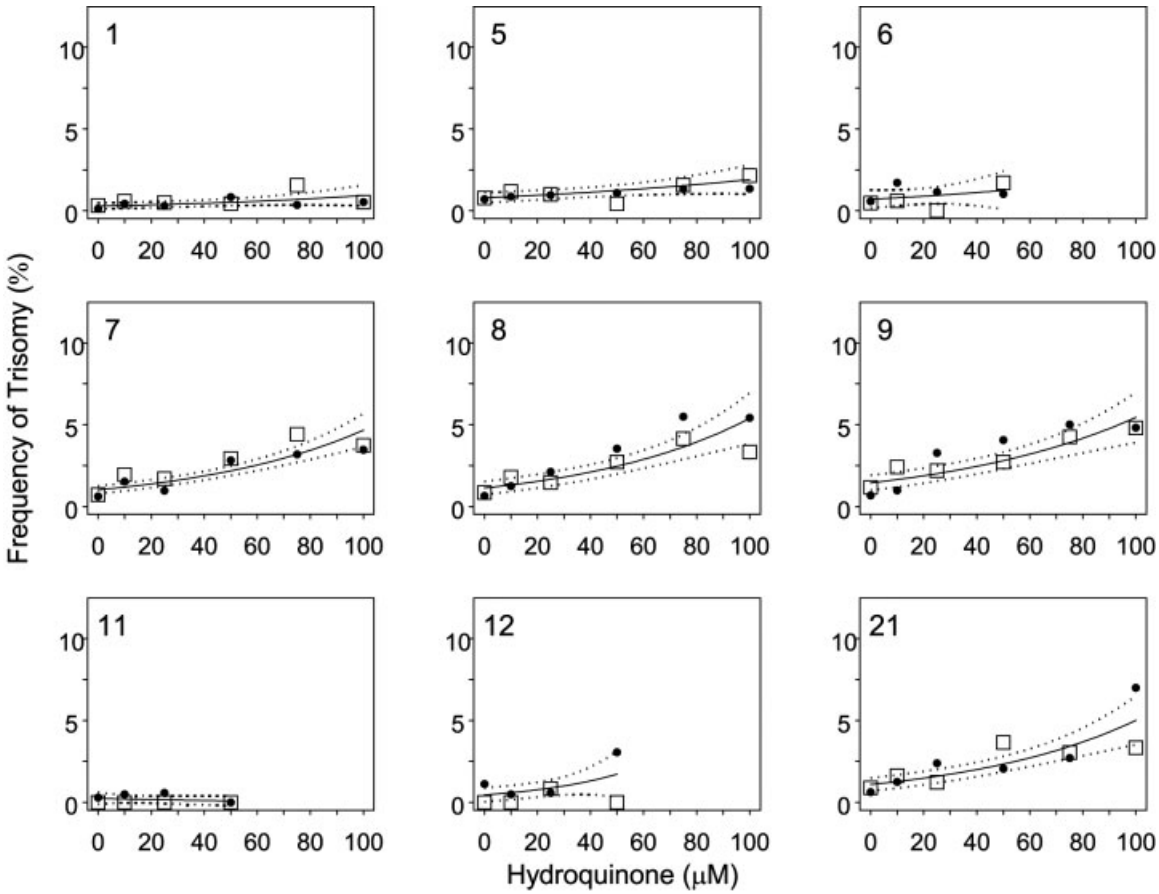

Fig. 3. Frequency of trisomy induced by HQ in chromosomes 1, 5, 6, 7, 8, 9, 11, 12, and 21. Mean values for trisomy of the nine chromosomes following treatment with 0, 10, 25, 50, 75, and 100 μM HQ in cultured human lymphocytes are presented for each blood donor (as open squares and filled circles). Each donor's blood was cultured in three separate experiments. The graphs represent the best log-linear (or log-quadratic) fit to the actual raw data (solid line) and the 95% pointwise confidence intervals as dashed lines. Data for chromosomes 1, 5, 6, 7, 8, 9, 11, 12, and 21 are shown sequentially with the chromosome number indicated in the top left corner. Only six chromosomes (1, 5, 7, 8, 9, and 21) were examined at higher doses of HQ (75 and 100 μM) due to its cytotoxicity such that fewer cells were obtained.

tive fit of the Poisson model to an equivalent negative binomial model. All models were repeated adjusting for donors, experiments, and multiplicative interactions between each donor and dose and between each experiment and dose. Since there was no appreciable difference in the results of these sets of models, the results of the models that did not adjust for donors and experiments have been reported here.

Next, the dose-response relationship between aneuploidy frequency and HQ/BT level for each tested chromosome was examined separately. Poisson regression implies a log-linear model of the rate of aneuploidy vs. dose, specifically, $\log [E(Y/\text{dose})] = \beta_0 + \beta_1 \times \text{dose} + \beta_2 \times \text{dose}^2 + \ldots$, where Y is the aneuploidy frequency and E is the mean. For each chromosome, both a simple linear term for HQ/BT level (dose) and a linear plus quadratic term for HQ/BT level (dose + dose^2) were compared in the Poisson regression models. Both the likelihood ratio test and the relative Akaike information criterion (AIC) [Akaike, 1978] were used to decide whether the simple linear dose-response was sufficient to achieve the best fit to the data. If it was sufficient, the simple linear model was used. If not, the linear plus quadratic model was used (only for monosomy 7 and 12 induced by HQ). The mean aneuploidy frequencies (%) for each donor and the best log-linear (or log-quadratic) fitted lines with 95% pointwise confidence intervals (CIs) are presented in Figures 1, 3, 5, and 7. The IRRs, 95% CIs for IRRs, and corresponding *P* values are presented in Figures 2, 4, 6, and 8.

Finally, we tested the following *a priori* hypotheses: one, the dose-response for HQ/BT versus monosomy frequency was significantly more pronounced for chromosomes 5 and 7 than the remaining seven chromosomes; and two, the dose-response for HQ/BT versus trisomy frequency was significantly more pronounced for chromosome 8 than the remainder of the chromosomes tested. For the monosomy analyses, a dummy variable was included to indicate chromosome 5 or 7 (=1 if chromosome is 5 or 7; =0 if otherwise); likewise for the trisomy analyses, a dummy variable was included to indicate chromosome 8. The models included the main effect of HQ or BT dose, the chromosome dummy variable, and the multiplicative interaction of dose and the chromosome dummy variable. We report the estimated IRRs for a change in dose of 50 μM for all analyses, but this estimate is made using various dose ranges within the data (details shown in Table I). For instance, the estimated IRRs for a change in dose of 50 μM for HQ and monosomy are made using the full range of doses (0–100 μM) and then repeated using only the data on low dose ranges (0–25 μM) and extrapolated to a 50 μM change. For the HQ analyses over the range of 0–100 μM, chromosomes 6, 11, and 12 were excluded as the experiments for these chromosomes only included doses in the range of 0–50 μM. The likelihood ratio test of the null hypothesis that the interaction term coefficient is 0 is the test of equality of dose-response. Thus, when the *P* value is < 0.05, it indicates a significantly disparate dose-response.

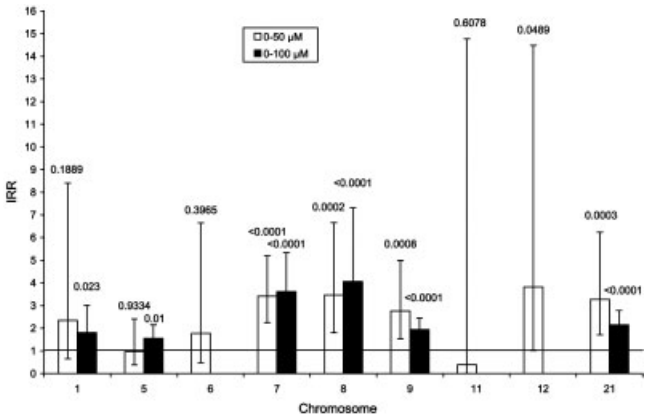

Fig. 4. Selective effect of HQ on trisomy of chromosomes 1, 5, 6, 7, 8, 9, 11, 12, and 21. The IRRs that describe how aneuploidy frequencies rise as HQ dose increases are shown for each chromosome tested over two dose ranges. Clear bars represent HQ treatments from 0 to 50 μM only, while shaded bars represent the HQ treatments that included the higher doses of 75 and 100 μM (for six chromosomes only). The error bars are shown as the 95% CIs and *P* values for dose-response are presented above them. IRRs, 95% CIs, and *P* values were calculated from Poisson regression models as described in text.

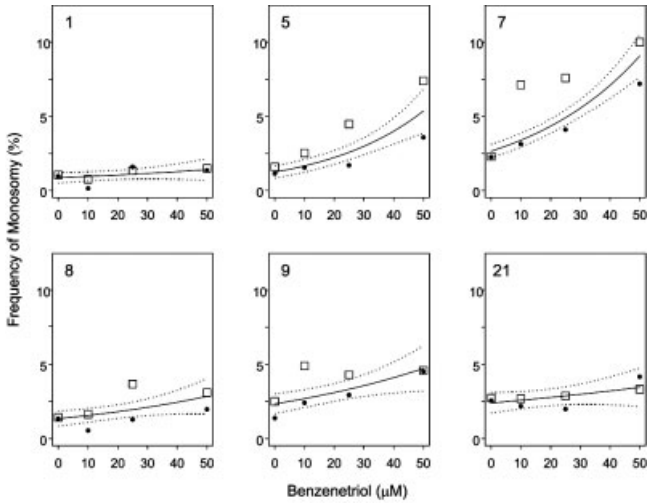

Fig. 5. Frequency of monosomy induced by BT in chromosomes 1, 5, 7, 8, 9, and 21. Mean values for monosomy of the six chromosomes tested following treatment with 0, 10, 25, and 50 μM BT in cultured human lymphocytes are presented for each blood donor (as open squares and filled circles). Each donor's blood was cultured in three separate experiments. The graphs represent the best log-linear (or log-quadratic) fit to the actual raw data (solid line) and the 95% pointwise confidence intervals as dashed lines. Data for chromosomes 1, 5, 7, 8, 9, and 21 are shown sequentially with the chromosome number indicated in the top left corner.

RESULTS

Effects of Hydroquinone on Aneusomy Levels

Exposure to HQ produced a dose-dependent increase in monosomy of all chromosomes tested, with the exception of chromosome 21 (Fig. 1). The lack of effect on chromosome 21 may be due to its being the smallest chromosome tested, resulting in high rates of artifactual loss in control metaphases. Highly significant effects were observed for chromosomes 5, 7, 9, and 11 ($P < 0.0001$), but the highest IRRs (> 3) were observed for chromosomes 5, 6, and 12 (Fig. 2). When we looked at monosomy of 5 and 7 together in comparison to monosomy of other chromosomes, we found that HQ had a more profound effect on monosomy 5 and 7 than on other chromosomes over all concentrations ($P = 0.0025$) and at low levels of exposure (0–25 μM; $P = 0.029$; Table I). This result is in agreement with our *a priori* hypothesis that a greater effect would be observed for chromosomes 5 and 7 than the other chromosomes.

HQ also induced trisomy in dose-dependent fashion (Fig. 3), with the most profound effects being on chromosomes 7, 8, 9, and 21 ($P < 0.0001$ for 0–100 μM dose range; Fig. 4). The IRRs for chromosomes 7, 8, 12, and 21 all exceeded 3.0 (Fig. 4), but the increase in trisomy 12 was of borderline significance ($P = 0.0489$) and with a wide 95% confidence interval. Thus, HQ has the most profound effects on trisomy of chromosomes 7, 8, and 21, but when we determined whether HQ had greater effect on trisomy 8 than on trisomy of other chromosomes, we found no statistical difference (Table I), probably because of its significant effects on chromosomes 7 and 21. The trisomy-inducing effects of HQ are therefore selective but not unique to chromosome 8.

By examining the IRRs, one can also see that the effects of HQ are more profound at low doses (0–25 μM) than over the whole dose range up to 100 μM for all chromosomes, but especially for monosomy 5 and 7 and trisomy 8 (Table I).

Effects of 1,2,4-Benzenetriol on Aneusomy Levels

We studied the effects of BT on six of the nine chromosomes we examined after exposure to HQ. BT produced a dose-dependent increase in monosomy of chromosomes 5, 7, 8, and 9 but not of chromosomes 1 and 21 ($P > 0.05$; Figs. 5 and 6). Chromosomes 5 and 7 were the most sensitive to BT-induced loss, both having IRRs > 3.0 for monosomy induction and *P* values less than 0.0001 (Fig. 6). Not surprisingly, when we looked at monosomy of chromosomes 5 and 7 together in comparison to monosomy of other chromosomes, we found that BT had a more profound effect on monosomy 5 and 7 than on other chromosomes over all concentrations ($P < 0.0001$) and at low levels of exposure (0–25 μM; $P = 0.0076$; Table I). As for HQ, this result is in agreement with our *a priori* hypothesis that a greater effect would be observed on chromosomes 5 and 7 than on other chromosomes.

BT induced trisomy of all six chromosomes in a dose-dependent fashion (Fig. 7). The highest IRR was actually for

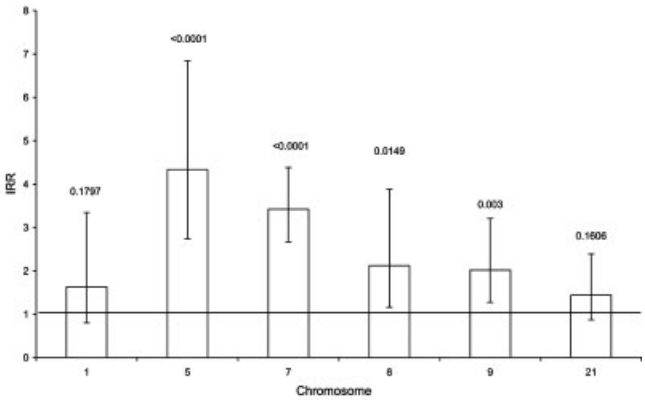

Fig. 6. Selective effect of BT on monosomy of chromosomes 1, 5, 7, 8, 9, and 21. The IRRs that describe how aneuploidy frequencies rise as BT dose increases are shown as clear bars for chromosomes 1, 5, 7, 8, 9, and 21. The BT treatments are from 0 to 50 μM only. The error bars are shown as the 95% CIs and *P* values for dose-response are presented above them. IRRs, 95% CIs, and *P* values were calculated from Poisson regression models as described in text.

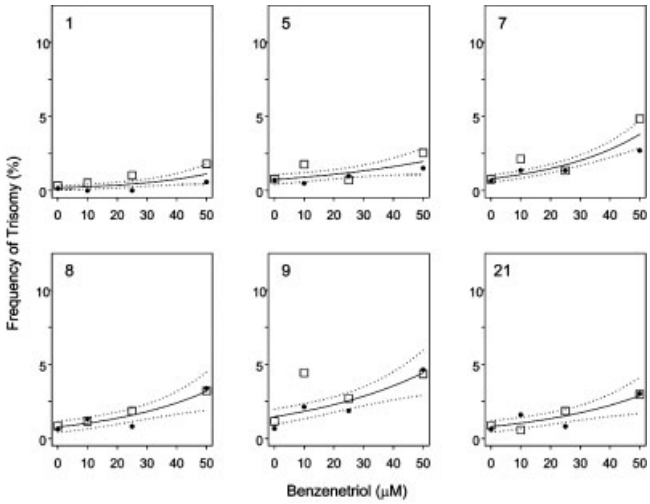

Fig. 7. Frequency of trisomy induced by BT in chromosomes 1, 5, 7, 8, 9, and 21. Mean values for trisomy of the six chromosomes tested following treatment with 0, 10, 25, and 50 μM BT in cultured human lymphocytes are presented for each blood donor (as open squares and filled circles). Each donor's blood was cultured in three separate experiments. The graphs represent the best log-linear (or log-quadratic) fit to the actual raw data (solid line) and the 95% pointwise confidence intervals as dashed lines. Data for chromosomes 1, 5, 7, 8, 9, and 21 are shown sequentially with the chromosome number indicated in the top left corner.

trisomy of chromosome 1, but the confidence intervals were also the largest (Fig. 8). In general, there was no discernible difference in trisomy induction among the six chromosomes, with all *P* values being less than 0.005 (Fig. 8). Further, trisomy 8 was not selectively induced by BT in comparison to the other five chromosomes tested ($P > 0.7$; Table I), which was similar to HQ. Thus, the trisomy-inducing effects of BT are not selective, in contrast to its selective monosomy-inducing effects on chromosomes 5 and 7.

Finally, we found no statistical evidence of overdispersion for any of the outcomes and none of the likelihood ratio tests comparing Poisson and negative binomial regression were significant.

DISCUSSION

Benzene is an established cause of human leukemia [Tomatis, 1982], but its precise mechanism of action is unknown. It is metabolized in the liver to its primary metabolite, phenol, which is hydroxylated to HQ and BT. HQ and BT can be further oxidized in bone marrow to 1,4-benzoquinone and 2-hydroxy-benzoquinone, respectively, and are genotoxic as a result [Smith et al., 1989; Zhang et al., 1993, 1994; Ross, 1996]. In the present study, we have tested the ability of HQ and BT to produce aneusomy of up to nine chromosomes simultaneously. Our first *a priori* hypothesis was that HQ and/or BT would have a more profound effect on monosomy 5 and 7 than on other chromosomes. We found strong evidence in support of this hypothesis in our experiments in HQ- and BT-exposed peripheral blood lymphocytes. This suggests that HQ and BT selectively affect the ploidy status of chromosomes 5 and 7. For chromosome 5, this selective effect was specific for loss, as trisomy 5 was not selectively increased. For chromosome 7, however, both

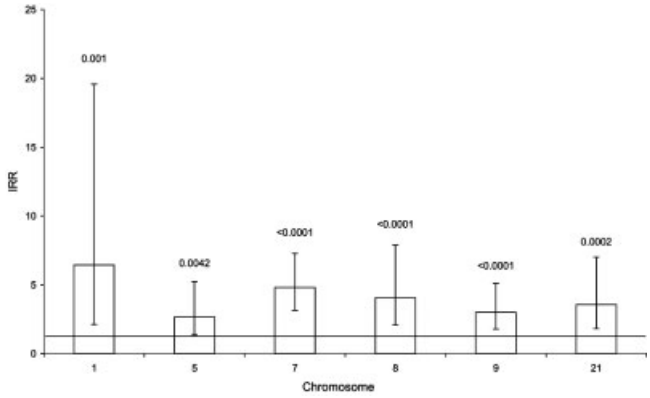

Fig. 8. Selective effect of BT trisomy of chromosomes 1, 5, 7, 8, 9, and 21. The IRRs that describe how aneuploidy frequencies rise as BT dose increases are shown as clear bars for chromosomes 1, 5, 7, 8, 9, and 21. The BT treatments are from 0 to 50 μM only. The error bars are shown as the 95% CIs and *P* values for dose-response are presented above them. IRRs, 95% CIs, and *P* values were calculated from Poisson regression models as described in text.

loss and gain were selectively increased in the presence of HQ, suggesting that the effects of HQ on chromosome 7 are related to nondisjunction. BT effects were, however, specific to loss of chromosomes 5 and 7.

The reasons why HQ and BT should have selective effects in causing the loss of chromosomes 5 and 7 remain unknown. Such selective loss is probably not caused by inhibition of microtubule assembly, a known effect of HQ and BT [Irons, 1985; Zhang et al., 1994], because effects

TABLE I. Test Results of Numerical Chromosomal Changes From the *a Priori* Hypothesis

	a Priori chromosomes[a]			Other chromosomes			Comparison		
	IRR1[b]	(95% CI)	*P*[b]	IRR0	(95% CI)	*P*	IRR1/IRR0	(95% CI)	*P*
Monosomy 5 and 7									
HQ (0–100 μM)[c]	1.87	(1.71–2.06)	< 0.0001	1.49	(1.32–1.67)	< 0.0001	1.26	(1.08–1.46)	0.0025
HQ (0–25 μM)	5.93	(3.59–9.80)	< 0.0001	2.58	(1.49–4.48)	0.0007	2.3	(1.09–4.84)	0.0287
BT (0–50 μM)	3.61	(2.90–4.50)	< 0.0001	1.79	(1.36–2.35)	< 0.0001	2.02	(1.42–2.87)	< 0.0001
BT (0–25 μM)	6.75	(4.01–11.39)	< 0.0001	2.28	(1.25–4.17)	0.007	2.96	(1.33–6.56)	0.0076
Trisomy 8									
HQ (0–100 μM)	2.19	(1.70–2.81)	< 0.0001	2.02	(1.81–2.26)	< 0.0001	1.08	(0.82–1.42)	0.582
HQ (0–25 μM)	4.98	(1.02–24.4)	0.0477	3.29	(1.75–6.17)	0.0002	1.51	(0.27–8.36)	0.6345
BT (0–50 μM)	4.06	(2.09–7.88)	< 0.0001	3.80	(2.93–4.93)	< 0.0001	1.07	(0.52–2.18)	0.858
BT (0–25 μM)	4.19	(0.81–21.7)	0.0874	3.22	(1.68–6.17)	0.0004	1.30	(0.22–7.63)	0.7698

[a]The *a priori* chromosomes 5, 7, and 8 were chosen from frequently observed aberrant chromosomes in leukemia patients.
[b]IRRs and *P* values were calculated from Poisson regression models as described in text and were bases on a 50 μM change in dose.
[c]Represents the dose range used to estimate the model.

on the mitotic spindle should be nonselective. Indeed, the well-established aneuploidogen, chloral hydrate, which inhibits spindle formation but does not damage DNA directly [Keller and Heck, 1988], did not cause selective aneuploidy in earlier studies performed in our laboratory [Xi et al., 1997]. However, because the process of chromosomal segregation is dependent on many different cellular components and is controlled by multiple signaling pathways, there are many ways that HQ and BT could be acting. HQ and BT could cause selective kinetochore detachment or breakage in the centromeric DNA region [Sgura et al., 2001]. Alternatively, they may alter the methylation status of specific centromeric DNAs [Esteller and Herman, 2002]. In both cases, selective chromosomes may fail to attach to the mitotic spindle and lag behind at anaphase. HQ and BT may also affect the expression and activities of the MAD and BUB spindle checkpoint proteins and/or the Aurora protein kinases [Ditchfield et al., 2003]. Support for the notion that chromosome lagging is responsible for the selective loss also comes from our finding that monosomy of chromosomes 5 and 7 caused by HQ and BT is not accompanied by a concomitant increase of the same magnitude in trisomy of these chromosomes (compare Figs. 1 with 3 and Figs. 5 with 7). Lack of incorporation of the lagging chromosome into the daughter nucleus would result in micronucleus formation and eventually monosomic and disomic, but not trisomic, nuclei. Thus, the selective effects are most apparent for monosomy induction. At present, we can only speculate as to the potential mechanisms for the selective effects of HQ and BT but this appears to be a fruitful direction for further studies.

Interestingly, while HQ and BT appeared to cause selective chromosome loss, specific effects on chromosome gain were far less apparent. Indeed, neither HQ nor BT caused selective gain of chromosome 8 to produce trisomy 8, a common clonal aberration in myeloid leukemia. Thus, if HQ and BT do selectively damage certain chromosomes in giving rise to benzene-induced leukemia, they do so by selectively causing loss of chromosomes 5 and 7, rather than gain (trisomy) of chromosome 8. This conclusion corroborates with what is known about chromosome changes in benzene-induced leukemia, where loss of chromosomes 5 and 7 appears to be the most common cytogenetic abnormality [Zhang et al., 2002a].

We are not the first to suggest that leukemogenic chemicals could be selective in affecting specific chromosomes. Indeed, Reeves and Margoles [1974] showed almost 30 years ago that chlorambucil-induced chromosome breakage was preferentially located on certain chromosomes. Similarly, Morad and El Zawahri [1977] showed a nonrandom distribution of cyclophoshamide-induced chromosome breaks. Then, in a series of classic studies, Mamuris et al. showed that the leukemogenic drug melphalan caused selective damage to chromosomes 5, 7, 11, and 17 both in vitro [Mamuris et al., 1989b] and in patients treated with this alkylating agent [Mamuris et al., 1989a, 1990]. Further, Sasiadek [1992] has suggested that benzene causes more breaks in certain chromosomes than in others. All of the above studies focus on the nonrandom distribution of chromosome breaks rather than selective aneuploidy. However, our findings of selective loss in chromosomes related to leukemogenesis and the above literature do suggest that leukemogenic chemicals, including benzene, could produce nonrandom chromosome damage that is key to the cancer process. Further studies with other chemicals are needed because different chemicals appear to cause different patterns of chromosome damage as our findings with HQ and BT reported here are quite different from those produced by the epoxide metabolites of butadiene [Xi et al., 1997].

Preliminary work on benzene's effects in exposed humans also suggests that it has selective effects on certain chromosomes. In a pilot study of 11 workers, we found that

benzene exposure (> 5 ppm time-weighted average) causes significant increases in loss of chromosomes 5, 6, 7, 8, 10, 12, 14, 16, 19, 21, and X (IRR from a Poisson regression model > 1.7; $P < 0.005$), with the strongest effects being on chromosomes 5, 6, 7, and 10 (IRR > 2.6) [Zhang et al., 2002b]. On the other hand, the monosomy levels of seven other chromosomes were unchanged in the exposed workers, with IRRs close to 1.0, suggesting that benzene has the capability of producing selective effects on certain chromosomes. Similarly, selective effects were also observed on trisomy induction, with chromosomes 8, 21, and 22 having the highest IRRs (IRR > 3; $P < 0.005$) [Zhang et al., 2002b]. These preliminary findings are being confirmed in a larger population of 88 workers, but the findings to date are in agreement with data from the in vitro studies presented here showing benzene metabolites cause selective aneusomy. Thus, HQ and BT may play an important role in benzene-induced leukemia by inducing the selective loss of chromosomes 5 and 7.

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