Direct and Indirect Clastogenic Activity of Anthracenedione in Chinese Hamster Ovary Cells

Larry J. Rosenberg and Walter N. Hittelman

ABSTRACT

The purpose of this study was to characterize the clastogenic activity of 1,4-dihydroxy-5,8-bis[(2-hydroxyethyl)amino]ethyl]amino]9,10-anthracenedione (NSC 301739), an antitumor compound now under clinical investigation. Chromosome damage in Chinese hamster ovary cells in G2 phase was assayed directly by the technique of premature chromosome condensation, and this damage was compared with the aberration levels determined when the G2 cells attained metaphase. 1,4-Dihydroxy-5,8-bis[(2-hydroxyethyl)amino]ethyl]amino]9,10-anthracenedione was observed to slow the progression of cells to mitosis and induce chromatid gaps, breaks, and exchanges directly in interphase cells. The frequency of gaps, breaks, and complete exchanges observed at metaphase were similar to those observed in G2 prematurely condensed chromosomes; however, the frequency of incomplete exchanges was increased in mitotic preparations. The additional exchanges appeared to result from chromosome stickiness occurring during chromosome condensation for metaphase. The chromosome attachments were strong and resulted in persistent chromosome bridges during anaphase. These results suggest that 1,4-dihydroxy-5,8-bis[(2-hydroxyethyl)amino]ethyl]amino]9,10-anthracenedione induces chromosome damage through both direct and indirect mechanisms.

INTRODUCTION

DHAQ (NSC 301739) is an aminoanthraquinone derivative that has potent antitumor activity. Although it is structurally similar to ADR, DHAQ is as or more effective than ADR in animal tumor models without causing dose-dependent cardiotoxicity (30). It is active against a wide spectrum of experimental tumors in vivo (11, 18, 29, 30) and is currently undergoing Phase I clinical trials (27, 28). DHAQ has been shown to bind to nucleic acids with high affinity and inhibit DNA and RNA synthesis, resulting in a cytostatic effect (5, 26). At low doses, it induces a G2 block in the cell cycle, which has been demonstrated by flow cytometry to occur 60 to 15 min prior to mitosis, i.e., during late G2 or early prophase (3, 26). Nevertheless, treatment of cells at any phase of the cell cycle can lead to reproductive cell death (5).

Previous studies with DHAQ or its nonhydroxylated analogue have demonstrated aberrant mitoses in Friend erythroleukemia cells and increased levels of sister chromatid exchange and chromosome breakage in CHO cells (1, 5, 19). The aberration studies, however, used treatments of 5 to 24 hr and scored only mitotic chromosomes for damage. The purpose of these studies was to better characterize the molecular basis of DHAQs clastogenic activity. In particular, we wished to (a) determine whether DHAQ could directly induce chromosome damage in interphase cells and (b) compare the levels of damage observed in interphase cells with that seen when these cells reached mitosis.

The premature chromosome condensation technique was used to visualize chromosome damage in G2 cells. This involves the fusion of interphase cells, using UV-inactivated Sendai virus, which results in the condensation of interphase chromatin into discrete chromosomes. Previous studies have shown that this technique allows a more sensitive measurement of chromosome damage in a cell population since (a) the G2 PCC are more extended than metaphase chromosomes and (b) chromosome aberration levels can also be determined in cells too damaged to reach mitosis (for reviews, see Refs. 9 and 12).

Our experiments show that DHAQ slows progression of CHO cells into mitosis and induces chromatid gaps, breaks, and exchanges directly in G2 cells after a 30-min exposure. Interestingly, a higher frequency of exchanges was observed in mitotic preparations of cells treated in G2 phase than were observed in G2 PCC immediately after treatment. This took the form of incomplete chromatid exchanges that appeared to result from chromosome stickiness and only became manifest at mitosis. These chromosome aberrations resulted in the formation of chromatid bridges and lagging chromosomes during anaphase. Thus, DHAQ appears to produce chromosome aberrations by both direct (immediate) and indirect (delayed) mechanisms.

MATERIALS AND METHODS

Cell Culture. HeLa and CHO cells were grown as monolayers in a humidified, 5% CO2 atmosphere at 37°C, using McCoy's modified Medium 5A (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% fetal calf serum (KC Biological, Lenexa, Kans.) and a penicillin-streptomycin mixture. Cells were subcultured with 0.05% trypsin (Worthington Biochemical Corp., Freehold, N. J.) and maintained in exponential growth prior to all experiments.

Cell Synchrony. In some experiments, cells were synchronized to increase the percentage of cells in G2. HeLa cells were incubated in 2.5 mM thymidine for 24 hr, followed by release into fresh medium for an additional 9 hr. To obtain CHO cells in G2, cells were incubated in 7.5 mM thymidine for 12 hr and then released for 7 to 8 hr. Mitotic CHO cells for PCC induction were obtained by incubating cultures in medium containing Colcemid (0.25 μg/ml; Ciba Pharmaceutical Co., Summit, N. J.) for 3.5 hr, followed by selective detachment. This resulted in a mitotic index of ≥95%.

1 Supported in part by NIH Grants CA 14528, CA 27931 and CA 28153 from the National Cancer Institute.
2 Rosalie B. Hite Predoctoral Fellow. To whom requests for reprints should be addressed.

The abbreviations used are DHAQ, 1,4-dihydroxy-5,8-bis[(2-hydroxyethyl)amino]ethyl]amino]9,10-anthracenedione, NSC 301739; ADR, Adriamycin; CHO, Chinese hamster ovary; PCC, prematurely condensed chromosomes; Con A, concanavalin A.

Received December 13, 1982; accepted March 29, 1983.
DHAQ. DHAQ was kindly provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md., as a 2-µg/ml solution in 0.8% NaCl solution and 0.2% sodium metabisulfite. The stock solution was stored sterile at 4° and protected from light. Working solutions of 100× the desired final concentration were prepared in medium without serum (incomplete medium) just before use and added to each dish to give the appropriate concentration.

Mitotic Inhibition Studies. To determine the effect of DHAQ on cell progression into mitosis, CHO cells were plated onto 60-mm dishes at a concentration of 1 × 10^6 cells/dish and allowed to grow for 24 hr. The appropriate concentration of DHAQ or an equal volume of incomplete medium was added to the dishes for 30 min, followed by 3 washes with fresh, prewarmed medium. Fresh medium was added, and the cells were incubated for an additional 30 min to allow cells already in mitosis to progress into G2. Colcemid was added (0.1 µg/ml), and dishes were harvested hourly using 0.05% trypsin. Cytocentrifuge preparations (Shandon Scientific Co., Sewickley, Pa.) were fixed in methanol and stained with Giemsa (Fisher Scientific Co., Fairlawn, N. J.). One thousand cells were scored for each time point at each dose to determine the mitotic index.

Chromosome Aberration Studies. Exponential cultures of CHO cells were treated with either DHAQ (0.1 µg/ml) or an equal volume of incomplete medium for 30 min at 37°. The dishes were rinsed 3 times with fresh medium, including a final wash for 5 min at 37°. Some dishes were processed immediately for PCC induction, while others were used for mitotic analysis. In the latter case, after DHAQ treatment the cells were incubated for an additional 30 min as above prior to 1.5-hr Colcemid accumulation of mitoses (0.1 µg/ml). This ensured that only cells in G2 at the time of treatment would be included in the analysis of mitotic chromosomes. The cells were then harvested, treated with hypotonic 0.7% sodium citrate, fixed 3 times in methanol:glacial acetic acid (3:1), and dropped onto wet slides. The slides were stained with Giemsa (Fisher).

The technique for PCC induction has been described in detail previously (7). Briefly, treated CHO interphase cells were collected and mixed 1:1 with CHO mitotic cells. After washing with Hanks' balanced salt solution the cells were fused using UV-inactivated Sendai virus. Slides for chromosome analysis were prepared as for mitotic preparations.

Scoring of Aberrations. Chromosome aberrations in CHO cells induced by DHAQ were scored blindly by using light microscopy and selecting well-spread figures from coded slides for analysis. Fifty G2 PCC and 100 metaphase spreads from each of control and treated cultures were examined. Aberrations were scored as chromatid gaps, breaks, or exchanges. Exchanges were classified as complete or incomplete interchanges (see below). Complete exchanges are those in which all four ends of the broken chromatids are reunited, while incomplete exchanges have one or two ends that fail to rejoin. Complex exchanges were frequent; these were broken down into pairs of incomplete interchanges to allow for easier interpretation of results. Fragments were considered as isochromatid breaks and were counted as 2 breaks. No single chromatid fragments were observed.

Anaphase Studies. To examine anaphase figures after DHAQ treatment, HeLa cell populations enriched in G2 CHO cells were exposed to DHAQ (0.1 µg/ml) for 30 min and then washed and incubated as described above. Mitotic cells were accumulated by a 2- to 3-hr nitrous oxide block at 37° (23), selectively detached, washed with fresh medium, and replated onto coverslips previously treated with Con A to increase cell attachment. The cells were incubated at 37° to allow anaphase to proceed, and sets of coverslips were harvested at various intervals between 60 and 90 min. The coverslips were fixed in 3:1 methanol:glacial acetic acid and later stained with Giemsa. Slides were scored blindly for lagging chromosomes, bridges, and multipolar divisions. Fifty to 100 cells were scored for each time point.

The Con A treatment of coverslips was used to increase cell attachment to the coverslip and to prevent cell loss upon fixation (6). For this procedure, 22- x 22-mm coverslips were placed in 35- x 10-mm plastic Petri dishes, and 0.8 ml of a 75-µg/ml solution of water-soluble carbodiimide (1-cyclohexyl-3-(2-morpholinopropyl)carbodiimide metho-p-toluene sulfonate, Sigma Chemical Co., St. Louis, Mo.) was added to each coverslip. Then, 0.8 ml of Con A (0.5 mg/ml; Grade IV; Sigma) was added. The dishes were maintained at room temperature for 2 hr with frequent shaking. This mixture was then poured off, and the dishes rinsed 3 times in phosphate-buffered saline (145 mM NaCl, 2 mM Na2HPO4, 2 mM NaH2PO4, pH 7.2) and stored in phosphate-buffered saline at 4° until ready for use.

RESULTS

Mitotic Inhibition. Initially, we performed experiments to determine the optimal dose of DHAQ for aberration studies in CHO cells. Such a dose should induce a minimum number of pyknotic cells and allow sufficient numbers of G2 cells to reach mitosis with scorable aberration frequencies. Exponential cells were treated with DHAQ (10, 1.0, 0.1, and 0 µg/ml) for 30 min at 37° and then washed 3 times with medium. After an additional 30-min incubation, Colcemid was added, and dishes were harvested hourly for cytocentrifuge preparations. As shown in Chart 1, DHAQ produced a rapid (<30 min) drop in the mitotic index at 10, 1.0, and 0.1 µg/ml. At 10 µg/ml, pyknosis of the chromatin was extensive, indicating cell death; less pyknosis was produced by 1.0 µg/ml, but a high level of chromosome shattering prevented accurate scoring for aberration frequencies. Little pyknosis was evident at 0.1 µg/ml, and chromosome aberrations were abundant but scorable. Thus, a dose of 0.1 µg/ml was used in the subsequent aberration experiments.

Chromosome Aberrations. The purpose of these experiments was to examine the immediate cytostatic effects of DHAQ on G2 CHO cells (visualized in G2 PCC) and compare this damage to that observed when these cells reached mitosis. DHAQ at 0.1 µg/ml induced chromatid gaps, breaks, and intrachanges that were immediately visible in the G2 PCC (Table 1). When the G2 cells were allowed to progress to mitosis, the frequency of gaps remained about the same, the breaks increased, and the frequency of incomplete exchanges increased nearly 3-fold. We obtained similar findings in another experiment using partially synchronized G2 cells, except that the frequency of chromatid breaks did not increase. The increase in the break frequency in Table 1 must be viewed with caution, however, considering the nature of these lesions (see below). Decondensation and uncoiling of mitotic chromosomes, reminiscent of treatment with actinomycin D (21), was also noted in chromosome spreads. This suggests that DHAQ interferes with chromosome condensation at mitosis.

We observed chromosome stickiness in the mitotic preparations, which appeared to produce a continuum of lesions ranging from thin fiber attachments to chromatid exchanges (Fig. 1). Qualitatively, there appeared to be a preference for telomeric interactions in these exchanges, giving rise to either incomplete exchanges or dicentrics. The dicentrics were presumably formed through a tight association of telomeres, since chromosome-type aberrations are not immediately formed in G2. Some breaks and gaps observed in mitotic chromosomes appeared to result indirectly from sticky adhesions between chromosomes (Fig. 1, a and b). This made accurate scoring of true breaks and gaps difficult. Very little stickiness was apparent in G2 PCC from treated cultures or in mitotic preparations from control cultures.

Anaphase Studies. We examined anaphase figures to deter-
mine whether the DHAQ-induced sticky lesions would have an
effect on chromosome segregation. Increased bridge formation
was expected since the drug caused complete and incomplete
exchanges. We wanted to know if the sticky lesions would also
contribute to bridge formation, indicating a strong attachment,
or were simply superficial and would come apart at anaphase.
We also wanted to see whether chromatid and sticky exchanges
could be distinguished at anaphase on the basis of thick and thin
anaphase bridges, respectively. Initially, we used CHO cells to
correlate the measured frequency of exchanges with bridge
frequency. However, CHO cells contain persistent nucleoli
throughout anaphase that are difficult to distinguish from true
chromatid bridges (10). HeLa cells do not have persistent nu-
cleoli, are easily synchronized, and also show similar chromo-
some stickiness with DHAQ (0.1 \( \mu \)g/ml) (data not shown).
Conversely, HeLa mitotic cells are very rounded and are easily
detached from the coverslip, so fixation of these cells in situ
would result in the loss of many mitotic cells. To circumvent
this problem, we collected HeLa mitotic cells with nitrous oxide (N2O)
following G2 drug treatment and plated them onto Con A-treated
coverslips. This resulted in rapid cell attachment and flattening
with minimal cell loss upon fixation of the coverslip.

Well-spread anaphase cells were scored for lagging chromo-
somes and bridges (Table 2). Since HeLa cells contain a signifi-
cant number of tripolar and tetrapolar mitoses with bridges and
lagging chromosomes after N2O treatment, only dipolar figures

![Chart 1: DHAQ mitotic inhibition.](chart)

**DISCUSSION**

The results described above show that DHAQ exerts a rapid
cytostatic effect on CHO cells, even after a 30-min exposure of
0.1 \( \mu \)g/ml. Drug treatment resulted in the immediate formation
of chromatid gaps, breaks, and exchanges that could be visuali-
zied in G2 PCC. Interestingly, at this dose, the interchange
frequency was seen to increase greatly from G2 to mitosis,
primarily due to an almost 3-fold increase in incomplete ex-
changes that seemed to result from chromosome stickiness.

At the dose of DHAQ used in these experiments, there was
no measurable decrease in the frequency of gaps or breaks from
G2 to mitosis. This finding could be explained by 3 distinct
hypotheses. First, DHAQ might induce irreparable chromosome
breaks or might itself inhibit chromosome break and gap repair.
Alternatively, DHAQ might remain bound to chromatin despite
continued washings and continue to induce DNA damage and
chromosome aberrations. If the rate of formation of these aber-
rations equaled the rate of repair, the aberration frequency would
appear to be unchanged from G2 to mitosis. A third possibility is
that the production of new breaks caused by the condensation of
chromosomes that contain sticky lesions may mask ongoing
repair. We cannot conclude from these experiments which of
these processes is occurring.

Table 2

<table>
<thead>
<tr>
<th>% of cells with</th>
<th>DHAQ (( \mu )g/ml)</th>
<th>Time after mitotic</th>
<th>Lagging chromosomes</th>
<th>Dipolar anaphase</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>60</td>
<td>9</td>
<td>74</td>
</tr>
<tr>
<td>0</td>
<td>70</td>
<td>6</td>
<td>9</td>
<td>88</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>85</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>60</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>70</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>0.1</td>
<td>+</td>
<td>85</td>
<td>5</td>
<td>57</td>
</tr>
<tr>
<td>0.1</td>
<td>+</td>
<td>60</td>
<td>60</td>
<td>58</td>
</tr>
<tr>
<td>0.1</td>
<td>+</td>
<td>70</td>
<td>61</td>
<td>48</td>
</tr>
<tr>
<td>0.1</td>
<td>+</td>
<td>85</td>
<td>48</td>
<td>83</td>
</tr>
</tbody>
</table>

were used for analysis. Although the N2O used to accumu-
litate mitoses slightly increased the frequency of multipolar anaphase
figures, it did not increase the proportion of bridges or lagging
chromosomes in dipolar anaplast figures. At 0.1 \( \mu \)g/ml DHAQ, there was an
increase in both lagging chromatid fragments and bridges relative
to control cultures. Drug-treated cells that entered anaphase last
exhibited both the highest bridge frequency and the highest
frequency of multipolar figures. This observation suggests that
either the most damaged cells were delayed in entry into ana-
phase or early G2 cells sustained more damage than late G2
cells. While the purpose of the anaphase experiments was to
distinguish bridges forming due to true chromatid exchanges
from those due to sticky chromosomes, it was not possible to
discriminate between thin and thick anaphase bridges due to
stretching and thinning of the chromosome fibers during ana-
phase chromosome movement.

<table>
<thead>
<tr>
<th>Spontaneous and induced aberrations in CHO G2 PCC and mitotic chromosomes</th>
<th>Aberrations/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaps</td>
<td>Breaks</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Control G2 PCC</td>
<td>0.10</td>
</tr>
<tr>
<td>DHAQ G2 PCC</td>
<td>1.28</td>
</tr>
<tr>
<td>Control mitotic</td>
<td>0.13</td>
</tr>
<tr>
<td>DHAQ mitotic</td>
<td>1.21</td>
</tr>
</tbody>
</table>

Table 1

**Table 2**

<table>
<thead>
<tr>
<th>Spontaneous and induced aberrations in HeLa anaphase figures</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa cells partially synchronized to G2 were incubated 30 min in the presence or absence of DHAQ (0.1 ( \mu )g/ml), washed free of drug, and allowed to proceed to mitosis. Mitotic cells were collected either by selective detachment or by N2O treatment, replated onto Con A-treated coverslips, and allowed to progress to anaphase. Coverslips were fixed at various intervals in methanol/acetic acid (3:1). Fifty to 100 cells were scored per sample.</td>
</tr>
<tr>
<td>% of cells with</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>DHAQ (( \mu )g/ml)</td>
</tr>
<tr>
<td>CONTROL</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.1</td>
</tr>
<tr>
<td>0.1</td>
</tr>
</tbody>
</table>
A high proportion of treated HeLa cells exhibited bridges at anaphase, presumably due to chromatid exchanges. It was not possible to discriminate between bridges caused by sticky lesions and those caused by chromatid exchanges. However, a large fraction of mitotic interchanges in CHO cells are due to incomplete exchanges, many of which seem to arise from sticky lesions. Since incomplete exchanges are also seen in HeLa cells and the bridge frequency in HeLa cells was 5-fold greater than controls, at least some of these sticky lesions probably contribute to bridge formation in HeLa cells. This suggests that such sticky lesions are not merely superficial adhesions but are steadfast chromosomal connections.

The effects of DHAQ on CHO cells are very similar to those seen with ADR. Both drugs induce a G2 block, and both can inhibit DNA and RNA synthesis in vitro. More pertinent, ADR not only induces gaps and breaks in CHO chromosomes but also causes the same kind of sticky exchanges seen with DHAQ. At high ADR doses, aberrations were observed to increase with time in both G2 PCC and in mitotic preparations. However, at lower doses, ADR-induced chromatid breaks could be repaired (8). It was not tested whether a similar observation could be made with DHAQ. Au et al. have shown, however, that DHAQ is the more potent clastogen, inducing more aberrations and sister chromatid exchange than ADR at equimolar concentrations (1).

The similar action of these 2 compounds probably stems from their common anthracycline structures (15). DHAQ, like ADR, is a planar heterocyclic compound but is bisubstituted at positions 1 and 4 of an aromatic ring with alkylamino side chains. Since the side groups of DHAQ and ADR are very different, intercalation of part of the aromatic system into DNA could explain some of their similar effects. The proposition of intercalation of DHAQ into DNA is further supported by the findings that it stabilizes DNA against thermal denaturation (11, 22), unwinds SV40 DNA (13), and competitively binds with the intercalating agent ethidium bromide (22). Intercalation could account for the inhibition of DNA and RNA synthesis previously observed. However, not all intercalating agents are clastogenic (e.g., propidium iodide), so intercalation is not in itself sufficient to account for the breakage induced by DHAQ. Kapuscinski et al. (13) have suggested that the alkylamino residues of DHAQ may participate in ionic interactions with DNA. Others have shown that this portion of the molecule is critical for antitumor activity in vivo (17, 29, 30). Since chromosome damage is closely correlated with reproductive cell death, the alkylamino residues might be responsible for direct DNA and chromosome breakage. In support of this direct mechanism, DHAQ has been shown to produce single- and double-strand breaks in SV40 DNA (25). Ionic binding of DHAQ to DNA might occur alone or simultaneously with intercalation (13).

The chromosome stickiness seen with DHAQ and ADR might result from intercalation of the ring system into DNA. Other compounds that cause either chromosome stickiness or "subchromatid" lesions (e.g., ethidium bromide, actinomycin D, 8-ethoxycaffeine, coumarin) are known to intercalate into DNA or have an aromatic system or both (14, 16, 20, 21). Chromosome stickiness is produced when ADR or actinomycin D is added to cells in early prophase or in G2, as is shown here for DHAQ. The stickiness does not become apparent until mitosis, presumably when chromosome condensation during prophase exerts enough tension to stretch out fibrous connections that form between chromosomes. Since stickiness is not observed in G2 PCC, and since ADR and actinomycin D produce stickiness only in mitotic chromosomes after cells in G2 are treated, it is postulated that chromosome condensation during mitosis is necessary for these sticky lesions to arise. Evidently, the condensation that occurs to produce G2 PCC is not great enough to make stickiness apparent. It is not known why telomere regions of the chromosomes are more sensitive to this reaction.

Based on our observations and the proposed mechanism for the interaction of DHAQ with DNA, one can make some hypotheses concerning the molecular basis for these indirect aberrations. The folded-fiber models of Du Praw (4), Bahr (2), and Mullinger and Johnson (17) portray chromosomes as composed of longitudinal and looping fibers. These loops probably lie adjacent to other loops within the interphase nucleus. At prophase, the loops would be folded and retracted as the chromosomes condense. Intercalation of DHAQ into DNA might result in an uncoiling or rearrangement of chromatin loops. These loops would be free to interact with other such loops on the same or on different chromosomes. The degree of entanglement of chromatin fibers might determine the type of aberration observed at mitosis. If a small amount of chromatins becomes stretched out as the chromosomes condense away from one another during prophase, this would produce at metaphase 2 separated chromosomes connected by a thin fiber, as is often seen in mitotic chromosomes (Fig. 1a). Not all of these fibers would necessarily survive the fixation process. A stronger fibrous connection that resists stretching might cause one of the attached chromatids to break. This type of chromatid break is shown in Fig. 1b. Conversely, if the connection is strong and does not break or stretch upon condensation, an apparent incomplete exchange figure would be produced (Fig. 1, c and d). It is not possible to differentiate between this latter type of sticky exchange and a "true" exchange involving a recombination of DNA. Some cases are obviously sticky, while others give the appearance of true exchanges.

In conclusion, DHAQ appears to induce chromosome damage through 2 general mechanisms. DHAQ directly induces chromatid breaks and exchanges as a result of its DNA-breaking activity, and this damage can be seen immediately in G2 PCC after a brief treatment. DHAQ also induces chromosome stickiness, probably resulting from its intercalation into DNA, which only becomes manifest as the cell condenses its chromosomes at mitosis. This stickiness can result in the induction of new chromatid breaks as well as incomplete chromatid exchanges.

ACKNOWLEDGMENTS

The authors wish to thank Josephine Neicherl for help in preparation of the manuscript.

REFERENCES


JULY 1983


**Fig. 1.** Aberrations produced in mitotic chromosomes of CHO cells following treatment in G2 phase with DHAQ (0.1 μg/ml). Numerous gaps and breaks are evident, and uncoiling can be seen in the longer chromosomes. a, sticky association between 2 chromatids (long arrow), dicentric formation (short arrow), and thin fiber connection (arrowhead); b, sticky break induced on longer chromosome at right (long arrow) with smaller dicentric at left (short arrow); c, complete (long arrow) and incomplete (short arrow) interchanges; d, numerous incomplete interchanges (long arrows) and localized decondensation (lower right). x 2600.
Direct and Indirect Clastogenic Activity of Anthracenedione in Chinese Hamster Ovary Cells

Larry J. Rosenberg and Walter N. Hittelman


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/43/7/3270

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.