Increased Induction of Sister Chromatid Exchange by Diethylstilbestrol in Lymphocytes from Pregnant and Premenopausal Women

Anna Hill² and Sheldon Wolff³

ABSTRACT

Cultures of whole blood from pregnant women (4 to 6 months), premenopausal women, postmenopausal women, and normal men were grown in the presence of varying concentrations (1 × 10⁻⁵ M, 2 × 10⁻⁵ M, and 4 × 10⁻⁵ M) of diethylstilbestrol (DES), a synthetic estrogen and known carcinogen, to see if it had sex-related cytogenetic effects. DES induced sister chromatid exchanges in lymphocytes from pregnant and premenopausal women but had only a small effect at the highest concentration (4 × 10⁻⁵ M) in lymphocytes from men and postmenopausal women. At all concentrations, the average number of sister chromatid exchanges was higher in lymphocytes from pregnant women than in those from premenopausal women. In lymphocytes from both a man and a pregnant woman, DES strongly inhibited cell proliferation in vitro. When lymphocytes from a man and a pregnant woman were cocultured in the presence of DES, only the lymphocytes of the woman responded with an increase in sister chromatid exchanges. This indicated that there is no interaction between DES and a factor present in the blood but that DES acts directly on each cell.

INTRODUCTION

Adenocarcinoma of the vagina in young women is highly correlated with in utero exposure to DES, a synthetic estrogen (7, 9). The mechanism of DES action has been examined in many in vitro systems used as short-term tests for carcinogens. In the Salmonella test for mutagenicity and presumptive carcinogenicity, DES did not induce mutations even in the presence of a metabolic activating system (6, 14, 19). In cultured mammalian cells, DES caused unscheduled DNA synthesis (13), a property associated with many carcinogens that affect DNA, but failed to cause cell transformation (23). At the chromosomal level, DES did not increase the SCE rate in CHO cells (1) but did increase SCEs in human fibroblasts (19). DES has been shown to induce chromosomal abnormalities such as polyploidy in CHO cells (10), mitotic chromatin nondisjunction in HeLa cells (18), and aneuploidy in mouse embryos (4), mouse bone marrow (5) and cultured human synovial cells (12). In vivo studies of mouse bone marrow showed that DES induced chromosomal aberrations but not SCEs (11). These same studies showed that female mice were more sensitive than male mice to DES, and DES strongly inhibits proliferation of lymphocytes from both men and pregnant women and can induce polyploidy in lymphocytes from pregnant women.

MATERIALS AND METHODS

To test whether hormonal differences might influence DES action in human lymphocytes, cultures of whole blood from pregnant women (4 to 6 months), premenopausal women, postmenopausal women, and normal men were grown in the presence of various concentrations (1 × 10⁻⁵ M, 2 × 10⁻⁵ M, and 4 × 10⁻⁵ M) of DES. None of the women in this study were exposed to synthetic estrogens. 5-Bromodeoxyuridine was present in the cultures so that harlequin chromosomes (17) in which sister chromatids stain differentially were produced. Whole blood (0.3 ml) from each individual was added to 5 ml of Roswell Park Memorial Institute Tissue Culture Medium 1640 containing 15% fetal calf serum, 1% phytohemagglutinin M, penicillin (100 units/ml), streptomycin (100 µg/ml), glucose (0.5%), and 5-bromo-2-deoxyuridine (20 µM). DES (Sigma Chemical Co.) dissolved in DMSO was added in 20-µl aliquots 1.5 hr after the beginning of culture. DMSO alone (20 µl) was added to the control cultures. Cultures were incubated at 37°C for 72 hr in complete darkness. Two hr before fixation, Colcemid (2 × 10⁻⁷ M final concentration) was added. Cells were collected by centrifugation, exposed to 0.075 M KCl for 15 min, and fixed 3 times in methanol:acetic acid (3:1). A concentrated cell suspension was dropped onto slides. Slides were stained by means of a modification of the FPG technique of Perry and Wolff (17). After being stained in Hoechst 33258 (5 µg/ml), slides were washed, mounted in buffer, placed on a 60°C hotplate, and exposed to a black light for 10 min (625 J/min from 2 BLB fluorescent bulbs). The coverslips were removed, and the slides were stained in 4% Giemsa for 10 min. SCEs were analyzed in 50 second-division metaphases for each point, and the data were expressed as the mean number of SCEs per cell ± S.E.

In another experiment, 0.15 ml of blood from a man and 0.15 ml of blood from a woman were added to each flask. Control cultures containing DMSO and 0.3 ml blood from the same man or the same woman were established at the same time. Slides from cocultured cells were made from cell suspensions and stained in 0.5% quinacline dihydrochloride for 7 min. The slides were washed for 2 min and destained in McIlvaine's citric acid:phosphate buffer, pH 5.5, for 10 min. They were then dried and mounted in 1:1 glycerol:Sorensen's buffer (15% M), pH 7.4, and examined by fluorescence microscopy (exciter filter BP 436 ± 5 nm; chromatic reflector 460; barrier filter LP 478) to determine whether or not the cell contained a Y chromosome. Coordinates for individual cells classified as coming from the man or the woman were taken. The coverslips were removed, and the slides were destained in 100% ethanol for 5 min. The slides were then stained with the FPG method as described above. The same cells were reex-
amined, and SCEs were analyzed in 50 second-division metaphases for each point. Slides from the control cultures of male and pregnant female blood were only stained by the FPG method. One thousand metaphases from these control cultures of lymphocytes from a man and a pregnant woman were examined at each DES concentration to determine which division (first, second, or third) the cells were undergoing (24). Chromosomes in first-division metaphases had both chromatids darkly stained. Chromosomes in second-division metaphases had one chromatid lightly stained and its sister chromatid darkly stained. Third-division cells had some chromosomes that were completely light staining and some chromosomes in which one sister chromatid was lightly stained and the other darkly stained. Metaphases were also screened for the presence of polyplody, and the data were expressed as percentage of the total number of metaphases.

RESULTS AND DISCUSSION

Table 1 shows that DES induced significant increases in SCEs in lymphocytes from pregnant and premenopausal women, while having a small but significant effect only at the highest concentration (4 \times 10^{-5} \text{M}) in lymphocytes from men and postmenopausal women. DES concentrations of 1 \times 10^{-4} \text{M} or higher resulted in cell death in all cases.

To determine whether responses from individuals in each class were heterogeneous, a \chi^2 test was performed for each class at each DES concentration. When the data were heterogeneous, each point was plotted individually. The curves represent lines drawn through the average yield of SCEs obtained at each point (Chart 1). Individual responses at all points for the men and postmenopausal women and at the low DES concentrations (1 \times 10^{-5} \text{M} and 2 \times 10^{-5} \text{M}) for premenopausal women were homogeneous (p > 0.05). However, at the high DES concentration in premenopausal women (4 \times 10^{-5} \text{M}) and at all DES doses in pregnant women, individual responses were significantly different from one another (p < 0.05).

For each DES treatment, the differences between the average number of SCEs in each class were analyzed by Student's t test. Each class was compared individually to all other classes to determine statistical significance. The dose response for induction of SCEs in the lymphocytes from men and postmenopausal women was statistically the same (p = 0.45) and was significantly lower than that of the lymphocytes from pregnant and premenopausal women (p < 0.001). Although the responses of lymphocytes from individual pregnant women were not homogeneous at all concentrations, the average response was consistently higher than that in lymphocytes from premenopausal women. It should be noted, however, that the average response in lymphocytes from pregnant women was significantly different from that in premenopausal women only at the 2 higher concentrations (p < 0.001) and not at the lowest concentration (p = 0.1).

**Table 1**

<table>
<thead>
<tr>
<th>Individual</th>
<th>Age (yr)</th>
<th>0^b</th>
<th>1 \times 10^{-5}</th>
<th>2 \times 10^{-5}</th>
<th>4 \times 10^{-5}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant 1</td>
<td>27</td>
<td>6.86 ± 0.36^{c}</td>
<td>9.68 ± 0.44</td>
<td>10.72 ± 0.46</td>
<td>11.58 ± 0.48</td>
</tr>
<tr>
<td>Pregnant 2</td>
<td>35</td>
<td>6.06 ± 0.35</td>
<td>8.42 ± 0.41</td>
<td>9.88 ± 0.44</td>
<td>9.70 ± 0.44</td>
</tr>
<tr>
<td>Pregnant 3</td>
<td>25</td>
<td>6.70 ± 0.36</td>
<td>10.48 ± 0.46</td>
<td>10.32 ± 0.45</td>
<td>12.32 ± 0.50</td>
</tr>
<tr>
<td>Pregnant 4</td>
<td>29</td>
<td>6.30 ± 0.35</td>
<td>8.74 ± 0.41</td>
<td>9.20 ± 0.43</td>
<td>10.20 ± 0.45</td>
</tr>
<tr>
<td>Premenopausal 1</td>
<td>24</td>
<td>6.04 ± 0.35</td>
<td>9.04 ± 0.42</td>
<td>8.56 ± 0.41</td>
<td>10.30 ± 0.45</td>
</tr>
<tr>
<td>Premenopausal 2</td>
<td>25</td>
<td>6.32 ± 0.36</td>
<td>8.60 ± 0.41</td>
<td>9.26 ± 0.43</td>
<td>8.54 ± 0.41</td>
</tr>
<tr>
<td>Premenopausal 3</td>
<td>27</td>
<td>6.92 ± 0.37</td>
<td>8.80 ± 0.42</td>
<td>8.68 ± 0.42</td>
<td>10.00 ± 0.45</td>
</tr>
<tr>
<td>Postmenopausal 1</td>
<td>59</td>
<td>6.78 ± 0.37</td>
<td>7.10 ± 0.38</td>
<td>7.32 ± 0.38</td>
<td>7.74 ± 0.39</td>
</tr>
<tr>
<td>Postmenopausal 2</td>
<td>60</td>
<td>6.42 ± 0.38</td>
<td>6.72 ± 0.37</td>
<td>6.46 ± 0.36</td>
<td>7.86 ± 0.40</td>
</tr>
<tr>
<td>Postmenopausal 3</td>
<td>75</td>
<td>6.24 ± 0.35</td>
<td>6.52 ± 0.36</td>
<td>7.00 ± 0.37</td>
<td>7.22 ± 0.38</td>
</tr>
<tr>
<td>Male 1</td>
<td>27</td>
<td>6.76 ± 0.37</td>
<td>7.26 ± 0.38</td>
<td>7.36 ± 0.38</td>
<td>7.46 ± 0.39</td>
</tr>
<tr>
<td>Male 2</td>
<td>24</td>
<td>6.50 ± 0.36</td>
<td>6.08 ± 0.35</td>
<td>6.78 ± 0.36</td>
<td>7.90 ± 0.40</td>
</tr>
<tr>
<td>Male 3</td>
<td>18</td>
<td>6.92 ± 0.37</td>
<td>6.38 ± 0.35</td>
<td>6.58 ± 0.36</td>
<td>7.16 ± 0.38</td>
</tr>
</tbody>
</table>

^a Fifty cells/point.
^b DES concentration (M).
^c Mean ± S.E.

Chart 1. Dose-response curve for induction of SCEs by DES in human lymphocytes from men and pregnant, premenopausal, and postmenopausal women. Data at each DES concentration from individuals within a class were examined by a \chi^2 test. When the data at a given concentration were homogeneous, they were pooled. Values are from lymphocytes from pregnant women (■), premenopausal women (□), postmenopausal women (○), and men (△). Point with bar, average of 3 individuals ± S.E.
Because men and postmenopausal women have significantly lower amounts of estrogens and progesterones than do pregnant and premenopausal women (22) and DES caused only a small increase in the number of SCEs in lymphocytes from either men or postmenopausal women, these data suggest that cells containing high levels of female hormones interact with DES to produce SCEs. The variability seen in individual responses of lymphocytes from premenopausal and pregnant women might be due to variability of estrogen and progesterone concentrations among the women. It should be noted that men have high concentrations of testosterone, whereas postmenopausal women do not. Because low SCE frequencies were seen in the presence as well as the absence of high testosterone concentrations, the data indicate that testosterone was not playing a protective role. Experiments to determine whether the DES effect is related to estrogen receptors are now in progress.

To ascertain whether the differences between the responses of lymphocytes from men and pregnant women were the result of intrinsic differences in the cells rather than a diffusible factor in women's blood that interacts with DES, lymphocytes from a man and pregnant woman were cocultured in the presence of increasing concentrations of DES. The induction of SCEs in these cocultured cells was compared to that of cells from the same man and woman cultured separately. The results from the woman's blood that was cultured separately are included in Table 1 (Pregnant 4). One of the same men (Male 1) was used as a source of blood for the coculture experiment. The results obtained from his blood cultured separately as a control did not differ from the previous results presented in Table 1. Coculturing cells did not change the response of the individual cells to DES (Chart 2), indicating that there is no interaction between DES and a factor present in the blood but that DES acts directly on each cell.

**DES-induced SCEs in the Lymphocytes of Pregnant Women**

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effects of DES on cell cycle and polyploidy</th>
<th>% of metaphases in 1st, 2nd, or 3rd cell division</th>
<th>% of polyploid metaphases</th>
</tr>
</thead>
<tbody>
<tr>
<td>DES concentration (M)</td>
<td>Man</td>
<td>Pregnant woman</td>
<td>Man</td>
</tr>
<tr>
<td>0</td>
<td>13.2</td>
<td>27.2</td>
<td>56.9</td>
</tr>
<tr>
<td>1 x 10^{-5}</td>
<td>13.6</td>
<td>35.3</td>
<td>51.1</td>
</tr>
<tr>
<td>2 x 10^{-5}</td>
<td>20.8</td>
<td>43.7</td>
<td>35.5</td>
</tr>
<tr>
<td>4 x 10^{-5}</td>
<td>42.2</td>
<td>50.6</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*One thousand cells/point.*

DES strongly affected the ability of lymphocytes from both the man and the pregnant woman to divide in vitro (Table 2). The number of cells dividing 3 times within 72 hr in culture is strongly reduced in both male and female lymphocytes. Division of lymphocytes is dependent upon at least 2 events, transformation into a blast cell by a mitogen and the length of the cell cycle once the cell is transformed (16). DES may be affecting one or both of these processes. Male and female lymphocytes both show similar cell proliferation effects, suggesting that DES enters both cell types equally well and that the ability of DES to induce SCEs in lymphocytes from pregnant women is not due to a preferential uptake of the drug.

We also screened the lymphocytes for polyploidy and found that 2 x 10^{-5} M and 4 x 10^{-5} M DES induced a low degree of polyploidy in the lymphocytes from a pregnant woman (Table 2). These results are qualitatively different from the results in CHO cells (10) where 80% of the metaphases were tetraploid 48 hr after 6 x 10^{-5} M DES treatment.

The plasma concentrations of DES reached in vivo depend on the biological half-life of the drug. When men were given DES doses of 38 to 43 mg, the biological half-life of DES in the plasma was found to be 2 to 3 days (15). The differential effects between men and pregnant women seen in vitro occur at DES concentrations similar to those expected to accumulate in women given DES to prevent spontaneous abortion. Pregnant women usually started with a low daily dose of 2.5 to 50 mg, which was increased every 2 weeks during pregnancy to a dose of 150 mg/day (8, 21). A single dose of 150 mg of DES in a woman weighing 50 kg is approximately equal to 10^{-5} M. It is clear that, taken in daily doses, DES could accumulate in the blood and exceed concentrations of 10^{-5} M.

The relation between mutagenicity and carcinogenicity (14) makes it tempting to speculate that the carcinogenicity of DES is linked to its ability to cause alterations in DNA. Although studies examining SCEs and mutations in mammalian cells show no constant correlation between the two (3), chemicals that induce SCEs also induce mutations. Our SCE data therefore indicate that DES or its metabolites are able to attack DNA and produce lesions, some of which most likely are mutagenic. In this regard, it should be noted that DES has been shown to rapidly enter the blood cells of pregnant mice (20) and that cultures of mouse fetal cells were able to metabolize DES into active forms that produced adducts in the DNA (2).

In summary, our results show that the ability of DES to induce SCEs in lymphocytes is related to the hormonal state of the individual and that cells from premenopausal women and, especially, pregnant women show an increased susceptibility to DES.
REFERENCES


Increased Induction of Sister Chromatid Exchange by Diethylstilbestrol in Lymphocytes from Pregnant and Premenopausal Women

Anna Hill and Sheldon Wolff


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/42/3/893

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.