Cytogenetic Studies of Peripheral Leukocytes following Surgery and Radiotherapy in Patients with Breast Carcinoma*

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Chromosome analysis were carried out on peripheral blood leukocytes of breast cancer patient during the irradiation therapy after unilateral simple mastectomy. The observations were made at intervals varying from one to 5 weeks during the therapy and one month after the completion of the treatment. During the first and second weeks of treatment normal metaphase was noted and during the 4th and 5th weeks, there were no mitotic figures from the cell population. The chromosomal aberrations found after 3 weeks of treatment were, 11% of simple chromatid breaks, 7% of chromatid interchanges (translocations) and 8% of fragments. One month after the completion of the course of treatment showed a return of mitosis and that total chromatid breaks had decreased to 5%.

Radiation effects on cell division and chromosome aberration are discussed.

Since Muller reported the mutagenic effects of ionizing irradiation in 1927 and the pioneering work of Karl Sax in the 1930's, chromosomal aberration studies have become one of the most thoroughly studied areas of radiology (Bender, 1957). Also during these 50 years, irradiation of localized portions of the body has been employed for the treatment of certain tumors and the incidence of various tumors in the irradiated individuals has been studied. Radiation doses received by these patients varied considerably in the size of the fractionation pattern, and the tissue volume exposed. Radiation therapy usually involves partial-body irradiation in which the exposure field is limited as much as possible to the tissue being treated. Ionizing irradiation exerts its effect at a subcellular level and is geographically restricted. However it would appear that the effect of irradiation on chromosomes is not limited to a specific chromosomal site, but can be observed in a wide distribution of breakage in the aggregate of chromosomes.

Several reports have shown that radiation causes chromosome damage. These studies have included patients with accidental (Bender and Gooch, 1962, 1963; Sugahara et al., 1967; Goh, 1968, 1973, 1975; Buckton et al., 1971; Brewen et al., 1972), diagnostic (Bloom and Tjio, 1964), and therapeutic exposure (Amerose and Baxter, 1965; Millard, 1965; Saenger et al., 1973) to radiation. The abnormal chromosomes have been seen in patients as

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soon as 30 minutes and as long as 20 years (Buckton et al., 1962; Buckton and Pike, 1964; Tamura et al, 1970, Goh, 1975) after exposure. It appears that radiation can cause chromosomal breaks in human somatic cells and may have a long-lasting effect (Goh, 1968).

Since, the recently developed chromosome banding techniques offer improved possibilities for the study of chromosome aberrations, the main purpose of the present investigation was to determine the magnitude of the chromosome aberration with G-banding patters from the peripheral blood culture of patients receiving immediate postoperative radiotherapy for carcinoma of the breast. Preferential location of X-ray exposure induced chromosome breakage in the R-band of human chromosomes in vitro was reported by Holmberg and Jonasson (1973).

**MATERIALS AND METHODS**

The patient was a 64-year-old female with infiltrating ductal cell carcinoma of the breast. She has been treated with a daily dose of 200 rads of X-ray over a period of 7 weeks to bring the total dose of 4,800 rads in each area over the left internal mammary, left chest wall and left drain site, and the cobal-60 gamma radiation given was a total dose of 5,000 rads to left supraclavicular area and 6,000 rads to post axilla a week after simple mastectomy.

Blood for a control culture was drawn from the patient before the radiotherapy. The other blood samples were obtained within 2 hours after the 2, 3, 4 and 5 week treatment sessions and one month after termination of therapy. Chromosome studies were made on cells cultured from the leukocyte component of the blood using a modification of the technique of Moorhead et al. (1960). Colcemid, 0.5ug/ml, was added for 2 hours prior to cell harvest. Hypotonic treatment with KCl solution (0.075 M), followed by fixation in 3:1 methanol-acetic acid. After air drying all slides were stained in Giemsa stain. The G-banding preparation were made by the area digestion technique from Shiraiishi and Yosida (1972). 100 metaphases were analyzed from each culture. Photomicrographs were made using a light green filter on an AO photomicroscope with Kodak high contrast copy film.

**RESULTS AND DISCUSSION**

After the first and second week treatments no changes in mitosis and no evidence of structural abnormality in chromosomes were noted. After the 3rd week treatment session, chromosome aberration was found and after the 4th to 5th week session, there were no mitotic figures from the cell population. One month after completion of therapy normal mitotic figures were noted. One hundred metaphases taken after the 3rd week treatment were analyzed for chromosomal damage. The modal chromosome number was 46 which was

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**FIG. 1.** Distinctic, translocation, fragments and chromatid breaks after the 3rd week radiation treatment.
Table 1. Percentage incident of aberrant metaphase after the 3rd week radiotherapy treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aberrant Metaphases</th>
<th>Dicentric</th>
<th>Translocation</th>
<th>Simple break</th>
<th>Fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3 weeks after</td>
<td>22</td>
<td>3</td>
<td>4</td>
<td>11</td>
<td>8</td>
</tr>
</tbody>
</table>

seen in 98% of the metaphases analyzed. Twenty-two aberrant metaphases were recorded. The breakage points were randomly distributed. The large number of aberrations found in this study were of the chromatid type and the others were dicentric, translocations and fragments. The breakage points were preferentially located in interband area of G-bands. Some of the chromosome aberrations are illustrated in Figure 1. Table 1 gives the incidence of abnormal metaphases and of the various types of gross abnormalities found in cells after the 3rd week treatments as contrasted to cells before treatment.

During the 4th and 5th weeks of therapy no metaphases were noted and this indicated that all the mature lymphocytes, which are most radiosensitive cells, could have been destroyed and other cells may produce the temporal mitotic inhibition or because the present radiotherapeutic doses are too high and that cells were inhibited in their division after 4 weeks of irradiation. A number of papers have been published on the effect of X-ray on cell division cycle and subsequent mitotic delay as a function of cell-age (Phillips and Tolmach, 1966; Whitmore et al., 1967). They indicated that in many cell systems, exposure to ionizing radiation gives rise to, among other effects, a temporary disappearance of mitotic figures from the cell population (mitotic inhibition).

The cells from one month after termination of therapy show that mitosis had returned to normal levels and simple chromatid breaks were seen in 5%. Three polyplody forms were noted during the counting and analysis of 1,000 cells. From a study of the chromosome damage persisting after X-ray therapy to the spinal axis for ankylosing spondylitis it is noted that chromosome aberration can be seen as long as 5 years after X-ray treatment (Buckton et al., 1962). However, radiobiological studies on other materials suggest that the abnormalities are likely to disappear after a relatively few cell divisions (White, 1935; Sax, 1941; Tamura et al., 1974). The present findings may suggest that ionizing irradiation exerts its effect at a subcellular level and is geographically restricted. Thus, X-ray dosage and the exposure field of the body give different effects of irradiation. In this regard, even chromosome aberration analysis can be used for biological radiation dosimetry from peripheral lymphocytes after partial body irradiation, condition of radiotherapy must be important matters for analysis.

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