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EARLY EFFECTS OF LOCALIZED SINGLE DOSES OF IONIZING RADIATION ON HUMAN BONE MARROW

by

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Only sporadic observations are available on the effect of localized irradiation on the human bone marrow. All have been carried out during and after repetitive therapeutic irradiations (GOSWITZ, ANDREWS & KNISELEY 1963, HUTAFF & BELDING 1955, LEHAR et coll. 1966, STEWART & DISCHE 1956, SYKES et coll. 1960). To our knowledge there are no reports on the effect of single localized doses of ionizing irradiation on the differential cell count of human bone marrow in the immediate hours and days following exposure. In order to supply this information, the quantitative and qualitative changes in irradiated bone marrow of cancer patients were studied as a function of dose with constant time (two days after irradiation), and then as a function of time (4 hours, 1, 2, 3 and 6 days) with constant dose (400 rad).

Material and Method. Patients with bronchogenic carcinoma scheduled to receive radiotherapy were considered for this investigation. Under study in our institution at present is the comparative therapeutic effect on bronchogenic carcinoma of daily treatments versus split weekly doses. Patients receive either

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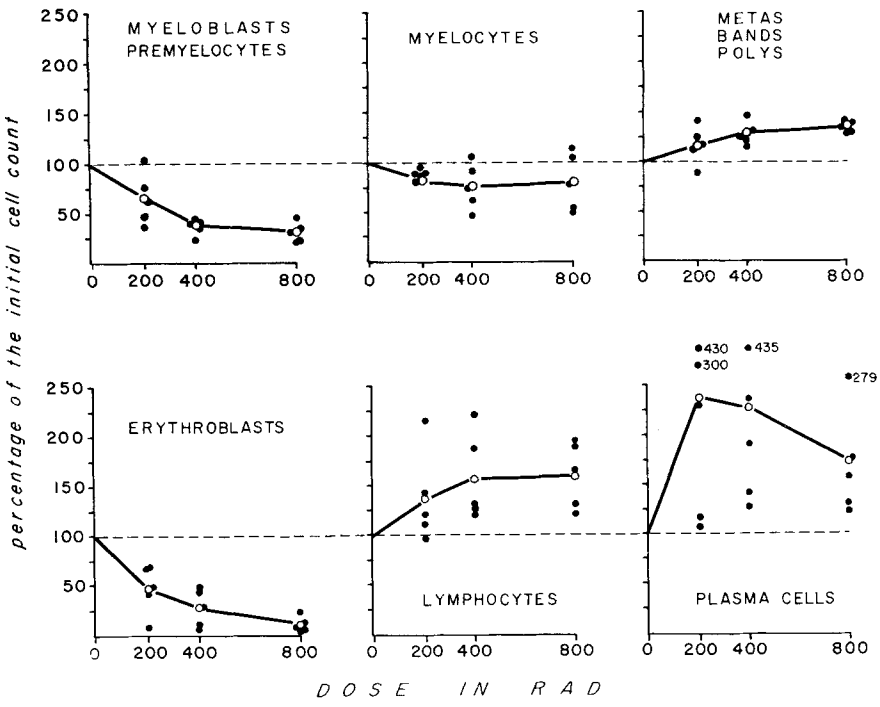

Fig. 1. Relative bone marrow changes two days after localized irradiation as a function of dose. Each point represents one patient; open circles show the mean values.

daily doses (four times a week) of 200 rad or single weekly doses of 400 or 800 rad, respectively, with a three-field technique, resulting in a tissue dose of 200, 400 or 800 rad ± 10 %, respectively to the center of the sternum. The physical factors of the irradiation are 300 kV, 4 mm Cu HVL, 50 cm target skin distance and 48 R/min dose rate.

The investigation was divided into two separate parts. Part I was designed to investigate the effect of localized irradiation as a function of dose. Fifteen patients were included in this study, five from each dose group. Two days after the initial exposure, permission was requested for a second bone marrow aspiration which the patients were told would be of experimental value only. In the second part of the study, which was designed to evaluate the effect of localized irradiation as a function of time, 20 additional patients, all from the 400 rad weekly split dose group, were included. Again, two aspirations were performed on each, one routinely before irradiation and another granted either 4 hours, 1, 3 or 6 days after the first set of treatments. At this point in the radiotherapy

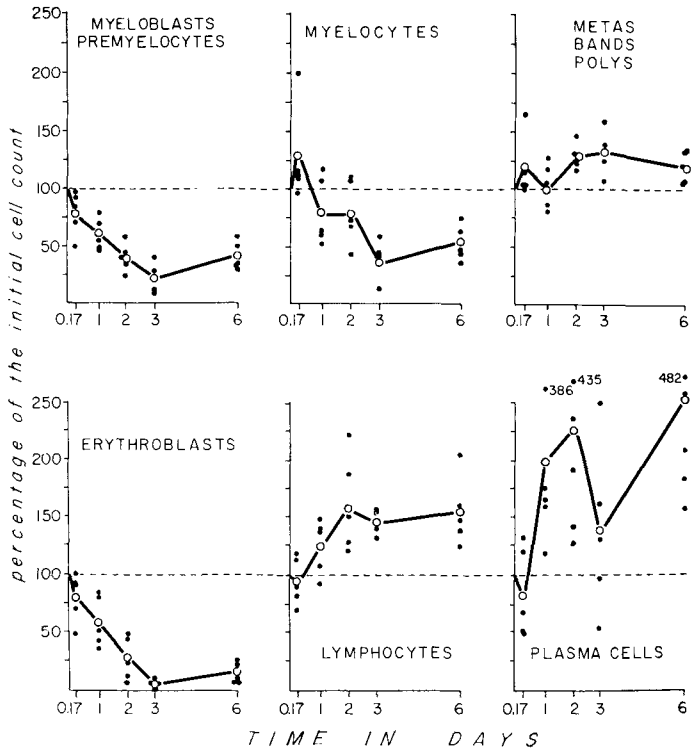

Fig. 2. Marrow changes after 400 rad as a function of time, expressed as a percentage of the initial count.

schedule each patient had received a sternal tissue dose of 400 rad. In addition, the data from the 400 rad group of the first study (examination after two days) were also included with these results. Included in this study were only patients who had no previous treatment, specifically no antineoplastic drugs. Furthermore, they must have shown no demonstrable clinical, laboratory or radiologic evidence of bone metastasis. In all cases studied, the routine initial bone marrow aspiration revealed a bone marrow cellularity and an erythroid/myeloid cell ratio within normal limits.

Results

The marrow changes in the three groups of five patients exposed to three doses of irradiation (200, 400, 800 rad), and examined after two days, are illustrated in Fig. 1. There is a drop in the myeloblast-premyelocyte group, which

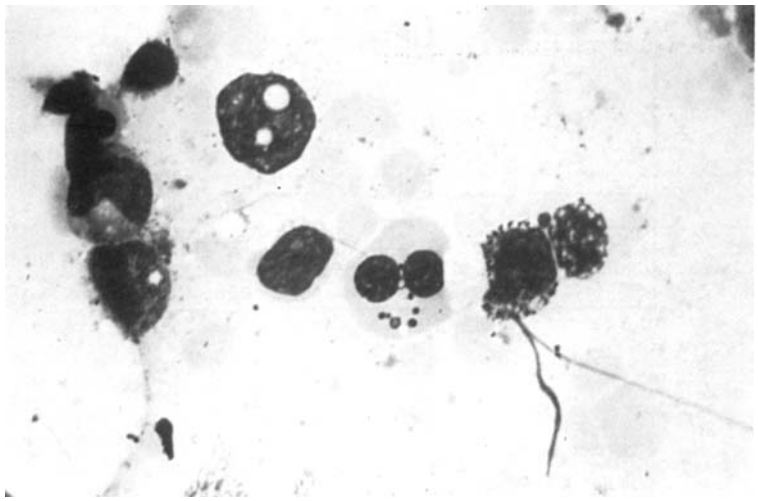

Fig. 3. Orthochromatic normoblasts have increased cell size, double nucleus connected by chromatin bridges and nuclear fragments in the cytoplasm. Bone marrow obtained 2 days after exposure to 400 rad. ×1025.

appears to be more marked at the higher dosages. There is also a drop in the mean relative percentage of the myelocytes, and a relative increase in the more mature myeloid cells, the latter again appearing more marked at the two higher dosages. The most marked changes, however, were observed in the erythroblasts. The mean relative drop in these cells grew larger with the roentgen dose, the mean reaching a low point of 10.8 % of the initial count after 800 rad. The relative percentage of lymphocytes rose strikingly, the mean being 137.3 for 200 rad, 157.1 for 400 rad, and 159.8 for 800 rad. The relative percentages of plasma cells followed a pattern similar to that of the lymphocytes; however they showed wider variations, due probably to the small initial count.

The changes produced by 400 rad as a function of time are recorded in Fig. 2. There was a relative drop in the myeloblast-premyelocyte group which was already evident at 4 hours, reached its lowest point at 3 days, and manifested some signs of recovery at 6 days. After an initial relative increase at 4 hours, the myelocytes followed a similar pattern but with a delay of about one day. The relative percentage of the maturing myeloid cells increased in 22 out of 25 patients and showed no clear dependency on the time of examination. The erythroblasts revealed the sharpest relative drop of any cell component. The drop began at 4 hours and as in the myeloid precursors, reached a very low point at three days (mean about 4 % of the initial count) and showed some signs of recovery at 6 days. We interpreted this later increase as a sign of intial

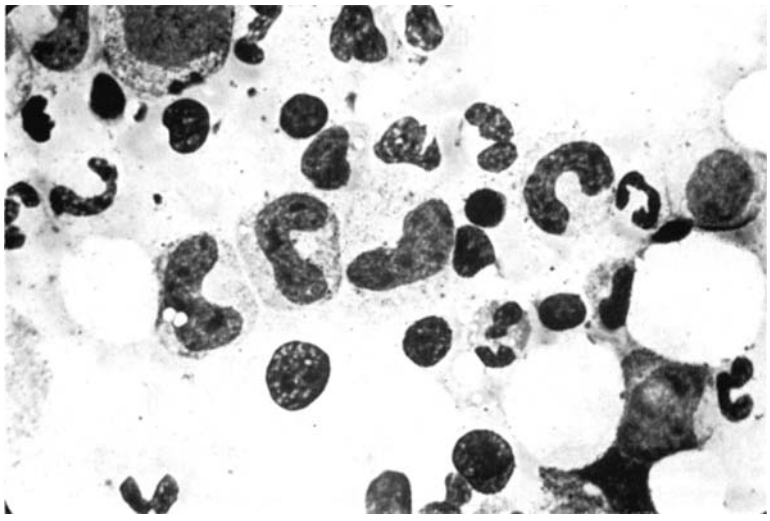

Fig. 4. 'Giant' bands observed in a smear obtained 3 days after exposure to 400 rad. Complete absence of erythroblasts and presence of several lymphocytes. ×785.

bone marrow regeneration because we also found in the 6-day smears basophilic and orthochromatic normoblasts which were not found in any of the smears obtained 2 and 3 days after irradiation. After an apparent slight initial drop at 4 hours, both the lymphocytes and plasma cells later rose and remained above the initial count throughout the entire investigation (6 days). As in the first experiment, the variations were wider in the plasma cells than in the lymphocytes.

No morphologic abnormalities were seen in the 4-hour smears. They were, however, seen in all smears taken 1, 2 and 3 days after irradiation. For the erythroblasts, these were characterized by an increase in cell size, disruption of the nuclear chromatin pattern, presence of nuclear fragments in the cytoplasm, and by double nuclei sometimes connected by small chromatin bridges (Fig. 3). The changes in the myeloid cells were characterized by marked increase in cellular size in some of the metamyelocytes and bands (Fig. 4), by the presence of toxic granulations in the cytoplasm and of finely dispersed chromatin in the nucleus, and by hypersegmentation of the polymorphonuclear cells. At 6 days, abnormalities were not noted in the erythroblasts but were still present in the myeloid series. No morphologic alterations could be detected at any time in the lymphocytes or plasma cells, although unrecognizably distorted and damaged cells were noted in all irradiated smears. It is interesting to note that two days after irradiation there appeared to be no connection between the number of

cells with morphologic abnormalities and the dose of irradiation received by the bone marrow.

Discussion

No attempt was made to determine the absolute bone marrow count, due to the well-known unreliability of such determinations on aspirated material. For this reason, although it is quite probable that all the observed percentage changes represent cell destruction, one cannot exclude the possibility of cell migration to or away from the irradiated areas. The erythroblasts and, to a lesser extent, the young cells of the myeloid series showed the most marked relative drop in the early days following irradiation. This is in the keeping with the well-established radiosensitivity of these elements (DENSTAD 1943). Moreover, the mean percentage changes in the erythroblasts seemed to be related to the dose of irradiation and to the time after irradiation, at least for the doses and times used. One can wonder whether repetitive bone marrow aspirations in the hours and immediate days after an accidental localized radiation would be of some use in approximating the degree of exposure. This, of course, still remains to be demonstrated.

The observation of a relative increase in the percentage of lymphocytes was quite surprising, considering the well-established high radiosensitivity of the blood lymphocytes. However, this observation in humans is not unique since it has been reported after repetitive localized irradiations (GOSWITZ et coll. 1963, HUTAFF & BELDING 1955, LEHAR et coll. 1966, STEWART & DISCHE 1956). Furthermore, similar findings have been described after whole body irradiation in humans and experimental animals. An increased percentage of lymphocytes was found in the bone marrow obtained after 3 to 5 weeks in humans exposed to the irradiation of the atomic bomb (OUGHTERSON & WARREN 1956). In individual cases, there were levels as high as 80 % and most of the cells appeared to be normal small lymphocytes. An absolute increase in the marrow lymphocyte count in mice 10 days after 400 R whole body irradiation has been observed by BRECHER et coll. (1948). Similarly, HARRIS (1956) found an increase in the absolute number of bone marrow lymphocytes in guinea pigs exposed 10 days previously to sublethal doses of whole body irradiation. From sequence of changes in the other bone marrow cell components, he found support for the hypothesis of Maximow, who first considered the lymphocyte to be a stem cell.

The observed relative increase of lymphocytes in our experiments could be the result of repopulation of the irradiated sternal area with normal lymphocytes migrating from non-irradiated sites. According to our observations this migration, if present, occurs promptly, being fully manifest already one day after irradiation. Another possibility is that the bone marrow lymphocytes are relatively more

radioresistant than other bone marrow cells and also more radioresistant than the blood lymphocytes. This hypothesis could be related to the findings of STEWART & DISCHE (1956), who noted a relative lymphocytosis in the bone marrow accompanied by marked lymphopenia in the blood of six patients with ankylosing spondylitis treated 1 to 189 days previously with localized irradiation. The suggested relative radioresistance of the marrow lymphocytes would give some support to the hypothesis, based on in vitro work, that there are two lymphocytic cell populations, one radiosensitive and one radioresistant (SCHREK & STEFANI 1964). Also, this observation would have some implication in organ transplantation. Whole body irradiation has been used for suppressing the immunologic defenses of organ recipients, i.e. to destroy the lymphocytes now well recognized to be the immunologic competent cell. It is obvious that if this cell is indeed more radioresistant than other bone marrow components, an attempt at immunosuppression by use of radiation would be comparatively ineffective.

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SUMMARY

The effect of localized single doses of ionizing irradiation on normal bone marrow of patients with bronchogenic carcinoma was studied. The studies were made as a function of dose and of time. Quantitative modifications consisted of a marked percentage decrease in the erythroblasts and a less marked percentage decrease in the precursors of the granulocytic series accompanied by a shift to the right. A noticeable relative increase in lymphocytes and plasma cells starting one day post-irradiation was observed.

ZUSAMMENFASSUNG

Der Effekt einer lokalisierten Einzel-Dosis ionisierender Strahlung auf das normale Knochenmark von Patienten mit bronchogenen Carcinomen wurde studiert. Die Untersuchungen wurden als Funktion der Dosis und der Zeit ausgeführt. Quantitative Veränderungen bestehen aus einem kräftigen prozentuellen Abfall der Erythroblasten und einem weniger ausgeprägten prozentuellen Abfall der Vorstufen der granulozytären Reihe, verbunden mit einer Rechtsverschiebung. Ein bemerkenswerter relativer Anstieg der Lymphozyten und Plasmazellen, der einen Tag nach der Bestrahlung einsetzte, wurde beobachtet.

RÉSUMÉ

Les auteurs ont étudié l'effet des doses uniques localisées d'irradiation ionisante sur la moelle osseuse normale de malades atteints de cancer bronchique. Ces études ont été faites en fonction de la dose et du temps. Les modifications quantitatives ont consisté en une dimi-

nution importante du pourcentage des érythroblastes et en une diminution moins importante des précurseurs de la série granulocytaire, accompagnée par une déviation vers la droite. Les auteurs ont observé une augmentation relative des lymphocytes et des cellules plasmocytaires commençant un jour après l'irradiation.

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