MITOTIC ACTIVITY AND CHROMOSOMAL ABERRATIONS IN THE REGENERATING RAT LIVER AFTER X-RAY IRRADIATION

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Most parenchymatous cells of the liver in adult animals are in the G_0-phase, which is insensitive to radiation. Most cells 4 h after partial hepatectomy (PH) enter the presynthetic G_1 phase [5, 6] and subsequent phase of interkinesis and mitosis synchronously. Ionizing radiation causes latent injury in the intact liver, manifested in the course of regeneration by delay of DNA synthesis and mitotic activity [2, 7, 9] and changes in the duration of certain phases of the cell cycle [10].

One of the best cytological indices for assessment of latent postradiation changes in liver cells is the frequency of chromosomal aberrations [3, 8, 13].

To determine more precisely the degree of injuries induced by radiation, changes in the mitotic index (MI) and the ratio between the number of metaphases and the number of prophases (M/P) and the frequency of chromosomal aberrations in postmetaphase were investigated for 21 days after PH in the regenerating liver of rats irradiated with x rays for exposures of 77.4, 154.8, and 253 mCi/kg body weight (300, 600, and 1000 R, respectively).

EXPERIMENTAL METHOD

Experiments were carried out on adult male Wistar albino rats with a mean body weight of 200 g. The animals were kept under ordinary animal house conditions with free access to food and water.

Immediately after irradiation for the exposures indicated above (on the TUR-T-250 therapeutic x-ray apparatus; 200 kV; 16 mA; dose rate 10.32 mCi/kg/min, i.e., 45 R/min) partial hepatectomy was performed on the animals in the usual way [11] and they were investigated between 18 h and 21 days after the operation (the animals were killed invariably between 6 and 8 a.m.) simultaneously with the control unirradiated animals. Squash preparations, stained by the Feulgen method, were prepared from the regenerating liver tissue. During the examination of 50,000-70,000 cells in each group all mitotic figures and chromosomal aberrations in postmetaphase were recorded. From these data MI (the number of mitotic figures per 1000 cells), M/P, and the number of cells with chromosomal aberrations as a percentage of the total number of postmetaphase figures found in the samples for analysis, were calculated.

The statistical significance of difference between the control and experiment was evaluated by the t-test.

EXPERIMENTAL RESULTS

Mitotic figures first appeared in appreciable numbers in the regenerating liver of the control animals after 24 h (Fig. 1). MI rose sharply to reach a maximum between 24 h and 30 h after PH, in agreement with results obtained by other workers [1, 7, 9]. During the subsequent period MI fell, and from the 7th day after the operation its values were close to those of the intact liver.

In irradiated animals of all three groups no mitotic figures were found before 24 h. Irradiation thus delayed the beginning of mitosis by about 6 h, in agreement with data in the

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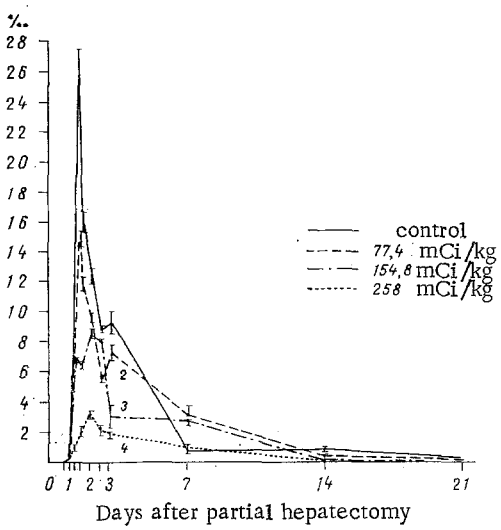


Fig. 1

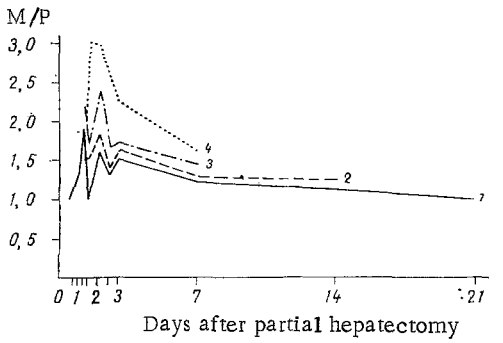


Fig. 2

literature [2]. The degree of inhibition of mitotic activity depended on exposure. After irradiation with an exposure of 77.4 mCi/kg the number of mitotic figures after 30 h was approximately half that in the regenerating liver of the control animals. The general trend of changes in MI until the 3rd day was similar to the control, but the values of MI were lower. On the following days the number of mitotic figures fell moderately, with the result that on the 7th day it exceeded the control level statistically significantly. From the 14th to the 21st days only single mitotic figures were observed.

In animals exposed to 154.8 and 258 mCi/kg the maximal number of mitoses was observed 48 h after PH, i.e., 18 h later than in unirradiated animals. During this period their number after exposure to 154.8 mCi/kg was approximately one-third, and after exposure to 258 mCi/kg approximately one-eighth of the maximal number of mitoses in the unirradiated control animals. During the 3rd day mitotic activity fell rapidly, and from the 7th day its character was the same as in animals irradiated with an exposure of 77.4 mCi/kg.

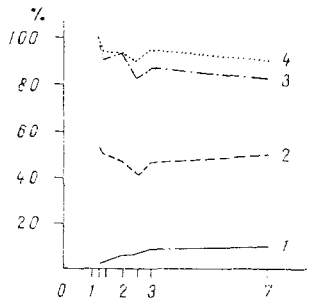
The results indicate that radiation mainly affects the first wave of mitosis, which in the control animals occurred before 30 h after PH. The difference between the values of MI in the irradiated and unirradiated animals at different times after PH varied considerably; for that reason observations made only at certain stages of regeneration cannot form the basis for a general conclusion on the degree of inhibition of mitotic activity. Measurements of the area of the figure indicate that a single exposure to 77.4 mCi/kg inhibited mitotic activity altogether by 15%, exposure to 154.8 mCi/kg to 39%, and exposure to 258 mCi/kg to 76%.

The relative number of metaphases to prophases in the regenerating liver of the control and irradiated animals during the first 3 days after PH fluctuated in waves (Fig. 2). After irradiation the values of M/P from the 36th hour after the operation were significantly higher than the control level; the degree of the differences depended on the dose rate. Before 30 h the ratio M/P was not calculated, for until that time mitotic figures did not appear in the irradiated animals. For the same reason, the M/P ratio was not calculated for the irradiated animals at the last times of observation.

The increase in M/P in bone marrow [12] and the regenerating liver after irradiation was explained by Karpfel by a change in the duration of one of these phases of the mitotic cycle: shortening of prophase or lengthening of metaphase.

During regeneration, however, M/P changed statistically significantly in the liver of the control, unirradiated animals also. This phenomenon is difficult to explain by a change in the duration of certain of these phases of the mitotic cycle. The effect of circadian rhythm was ruled out by ensuring that all the animals were killed at the same time of day (between 6 and 8 a.m.)

Changes in M/P in the regenerating liver of the control animals demonstrated the synchronized entry of the cells into the individual phases of the mitotic cycle, which continued until 48 h after the operation [4, 6], and they reflect the three gradually weakening waves of



Days after partial hepatectomy
Fig. 3

mitotic activity. Stabilization of M/P after the 3rd day points to completion of the synchronizing course. The fluctuation of the relative number of metaphases and prophases in the regenerating liver of the irradiated animals was evidently linked with definite synchronization, but under the influence of irradiation the fluctuation took place at a higher level. On the basis of the values of M/P and the MI curve it can be tentatively suggested that irradiation with a long exposure (258 mCi/kg) disturbs synchronization considerably.

For the first 3 days after PH the number of cells with chromosomal aberrations in the regenerating liver of the control animals rose gradually to 10% of the total number of postmetaphase figures (Fig. 3).

Until 30 h irradiation caused an increase in the number of aberrant figures, depending on the exposure, by 53, 97, and 100%, respectively. Until 60 h after PH some of the aberrant postmetaphases were eliminated.

After exposure to 77.4 mCi/kg, throughout the remaining period of increased proliferative activity chromosomal aberrations were found in 45-50% of postmetaphases.

The results differed only a little in animals irradiated with single exposures to 154.8 and 258 mCi/kg: In both cases the percentage of chromosomal aberrations at this time varied between 83 and 95%. These results agree with those of Stevenson and Curtis [13], who observed 80% of aberrant cells under similar conditions.

After a single exposure to 500 R, Karpfel [12] found up to 96% of aberrant cells in bone marrow. By contrast, Albert [8] found only 39% of aberrant postmetaphases in the regenerating mouse liver after a single exposure to irradiation in a dose of 700 R, and only 52% after exposure to 1000 R. The lower frequency of chromosomal aberrations in this case was due mainly to differences in the technique; regeneration of the liver was induced by CCl_4 and aberrations were counted in sections.

LITERATURE CITED

1. I. D. Belyaeva, Tsitologiya, No. 10, 1297 (1973).
2. N. Ya. Gil'yano and O. V. Malinovskii, Tsitologiya, No. 10, 1284 (1976).
3. N. Ya. Gil'yano and O. V. Malinovskii, Tsitologiya, No. 3, 367 (1977).
4. Yu. G. Grigor'ev, N. Ya. Savchenko, and N. I. Ryzhov, in: Problems in Radiobiology and the Biological Action of Cytostatic Preparations [in Russian], Vol. 3, Tomsk (1971), p. 5.
5. A. V. Zorin, N. A. Isanin, and A. Yu. Yakovlev, Tsitologiya, No. 7, 783 (1975).
6. O. V. Malinovskii, N. Ya. Mikhailova, N. Ya. Sigaleva, et al., Tsitologiya, No. 8, 1048 (1973).
7. A. M. Polishchuk, Tsitologiya, No. 6, 712 (1973).
8. M. D. Albert, J. Natl. Cancer Inst., 20, 309 (1958).
9. D. B. Cater, B. E. Holmes, and L. K. Mee, Acta Radiol., 46, 655 (1956).
10. J. I. Fabrikant, Radiat. Res., 31, 304 (1967).
11. G. M. Higgins and R. M. Anderson, Arch. Path., 12, 186 (1931).
12. B. Karpfel, Folia Biol. (Prague), 7, 129 (1961).
13. K. G. Stevenson and H. J. Curtis, Radiat. Res., 15, 774 (1961).