EFFECT OF X RAYS ON MITOSIS IN THE CORNEAL EPITHELIUM OF ALBINO MICE

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UDC 612.841.1.014.3:612.6]. 014.481.1

Mitotic activity was studied in the corneal epithelium of albino mice after total x-ray irradiation in the morning and evening. The experimental results show that marked diagonal fluctuations in mitotic activity continued to be observed in the corneal epithelium after x-ray irradiation whether in the morning or in the evening. A more marked antimitotic effect was observed after evening irradiation, evidently because of differences in the response of the cells to irradiation at different times of day. Whole-body x-ray irradiation of the animals led to the appearance of numerous pathological mitosis in the corneal epithelium, especially after morning irradiation.

Radiation damage to cells is accompanied by a disturbance of their mitotic activity. Investigations by the author and others [1, 7, 11, 13, 14] have shown that mitotic activity is depressed after irradiation and pathological mitosis appears [4, 11]. The problem arises whether the diurnal rhythm of mitotic cell division is maintained after whole-body irradiation of animals with x rays.

In connection with observations described in the literature [8, 13] it was also interesting to determine whether the response of cells to irradiation varies with the time of day. The experiments described below were carried out to study these problems.

EXPERIMENTAL

Experiments were carried out on noninbred albino mice aged 2.5 months which received whole-body x-ray irradiation in a single dose of 200 R from a type RUM-13 x-ray therapy apparatus (160 kV, 10 mA, filter Cu 0.5 mm + Al 1 mm, dose rate 54 R/min, skin-focus distance 30 cm, duration of irradiation 3 min). The animals were divided into two groups with 16 mice in each group. The mice of group 1 were irradiated at 9 A.M. and those of group 2 at 7 P.M. The animals were sacrificed at 9 A.M. and 7 P.M., 24 h after irradiation, in groups of eight mice at each time.

Control groups of mice were sacrificed simultaneously with the experimental groups (at 9 A.M. and 7 P.M., respectively).

Mitotic activity and the number of pathological mitoses were determined in 100 fields of vision; the number of undivided cells was recorded at the same time, after which the mitotic activity per thousand cells, the relative percentages of the individual phases of mitosis, the total number of pathological mitoses, and their separate forms were calculated.

The classification and technique of Alov [2] was used to determine the number of pathological mitoses and of their separate forms.

Statistical analysis of the results was carried out by the Fisher–Student method.

Department of Histology, Kursk Medical Institute. Laboratory of Cytology, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 11, pp. 90-93, November, 1972. Original article submitted March 25, 1972.

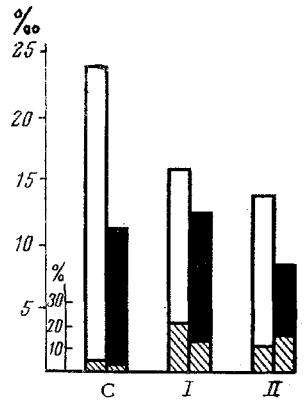

Fig. 1

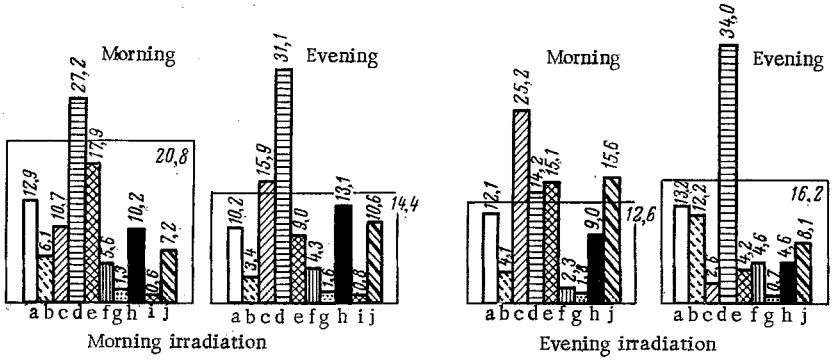

Fig. 2

Fig. 1. Mitotic activity and number of pathological mitoses in corneal epithelium of albino mice after x-ray irradiation in the morning and evening. Unshaded columns show mitotic activity in morning; black columns, mitotic activity in evening; obliquely shaded columns represent number of pathological mitoses. C) Control; I) irradiation in the morning; II) irradiation in the evening.

Fig. 2. Changes in number of pathological mitoses and in their separate forms after x-ray irradiation at different times of day. a) Bridges; b) bridges and fragments of chromosomes; c) retention of chromosomes and their fragments in metakinesis; d) deletion of chromosomes and their fragments during separation; e) dispersion of chromosomes in metaphase; three-group metaphase; g) hollow metaphases; h) asymmetrical mitosis; i) multipolar mitosis; j) mitosis. Large columns represent percent of pathological mitoses; small columns, relative percentages of various forms of pathological mitoses.

EXPERIMENTAL RESULTS

The results of these experiments showed that clear differences in mitotic activity were present in the cornea of the control animals at 9 A.M. and 7 P.M.

After total x-ray irradiation of the animals in a dose of 200 R the diurnal fluctuations in the number of mitoses still persisted regardless of the time of irradiation (at 9 A.M. and 7 P.M.; Fig. 1). However, the level of mitotic activity in the cornea of the experimental groups of animals was significantly lower than in the cornea of the control animals.

Persistence of the diurnal rhythm of mitoses in the adrenal cortex 6 h after whole-body x-ray irradiation in a dose of 800 R was observed by Truupyl'd [10]. Bulgak [3] also showed that the diurnal rhythm of mitoses is preserved in the regenerating liver at the height of radiation sickness.

Comparison of the diurnal fluctuations in mitoses in the cornea of albino mice irradiated in the morning with the changes in mitotic activity in the cornea of the animals irradiated in the evening showed that the fluctuations in mitotic activity were more clearly defined after evening irradiation (Fig. 1).

Analysis of the author's own observations and experimental results obtained by other writers [3, 5, 6, 9, 10] leads to the conclusion that the diurnal rhythm of mitoses is stable even after exposure to powerful antimitotic factors (radiation, total starvation, administration of adrenalin, myleran, etc.).

However, the time of day at which the particular factor acts has a substantial effect on the course of subsequent cell divisions.

Investigation of mitotic cell division in the corneal epithelium in the morning, 24 h after irradiation of the animals, revealed a significant difference in the number of mitoses by 31.2%.

When the animals were irradiated in the evening, mitotic activity in the corneal epithelium was reduced in the morning by a much greater degree, by 42.6% compared with the control ($P = 0.01$).

Analysis of mitotic activity in the evening (7 P.M.) shows that when the animals were irradiated in the morning the number of mitotically dividing cells was 10.9% higher than the control ($P = 0.05$), whereas irradiation in the evening led to a significant decrease of 22.7% in the number of mitoses.

Pathological mitoses were found extremely rarely (1.5-2%) in the corneal epithelium of the control animals. After x-ray irradiation their number rose sharply (Fig. 1). The largest number of pathological mitoses (20.8%) was found after morning irradiation in the group of animals sacrificed during the evening ($P = 0.001$). After evening irradiation of the animals the number of pathological mitoses in the corneal epithelium in the morning was 12.6%, i.e., 8.2% fewer than in mice irradiated with x rays in the morning.

The study of the pathology of mitosis in the evening indicates that the difference between the number of pathological mitoses in groups of animals irradiated in the morning and evening is small (1.8%) and is not significant ($P = 0.5$).

The results given in Fig. 2 show that the predominant forms of pathological mitosis are: 1) retention of the chromosomes and their fragments during separation; 2) retention of the chromosomes or their fragments in metakinesis; 3) dispersion of the chromosomes in metaphase; 4) chromosomal and chromatid bridges.

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