DIURNAL PERIODICITY OF MITOSIS IN MICE AFTER IRRADIATION WITH γ-RAYS

L. P. Kosichenko

Laboratory of Genetics (Head, Candidate of Medical Sciences N. P. Bochkov), Institute of Experimental Pathology and Therapy (Director, Dr. Med. Sci. B. A. Lapin) of the AMN SSSR, Sukhumi
(Presented by Active Member AMN SSSR A. V. Lebedinskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 55, No. 1, pp. 114-117, January 1963
Original article submitted March 20, 1962

The number of mitoses in the corneal epithelium, as in many other animal tissues, undergoes substantial changes in the course of the 24 hours [2, 8]. Several workers [3, 7] have shown that if animals are kept in artificial conditions of lighting (different from the natural alternation of light and darkness), the diurnal rhythm of mitotic activity is disturbed.

In order to compare the resistance of the diurnal rhythm of mitosis to the effects of certain external agents, causing essential changes in the condition of the organism, we investigated the diurnal rhythm of cell reproduction in animals exposed to ionizing radiation. There is very little information in the literature concerning the character of the diurnal periodicity of mitosis in animals after irradiation [1].

EXPERIMENTAL METHOD

Experiments were carried out on 100 male albino mice weighing 18-20 g. The test object was the corneal epithelium. The method of obtaining and preparing the histological material was described in detail previously [2].

One group of mice received whole-body irradiation with γ-rays. For this purpose the animals were placed in a cobalt γ-ray source with a charge of 4000 C of Co^{60}. The mice were kept in a hermetically sealed metal box, 65 cm in diameter, with a special air supply. In the working position, the box was submerged under water, where 8 rods with radiocobalt were arranged in a circle arround its circumference. The animals were irradiated in the mornings (8 to 8:30 A.M.).

Another group of animals acted as controls. The cages with the control animals were also placed in a container for 4-5 min at the same time of day, but were not irradiated. In this way the control and experimental animals were kept under identical conditions.

Two series of experiments with irradiation were carried out. In the first series the animals received a dose of radiation equal to 190 r and in the second a dose of 400 r. In both cases the animals were investigated on the 5th day after irradiation and sacrificed at intervals of 6 hours.

EXPERIMENTAL RESULTS

The results given in the table demonstrate the presence of a diurnal periodicity of mitosis in the corneal epithelium of the control mice. The pattern of the diurnal rhythm of mitosis in the corneal epithelium of the control animals of the first and second series of experiments was the same. The number of mitoses reached a maximum in the morning (8 A.M.) and a minimum in the evening (8 P.M.).

First series of experiments. The curve of the diurnal rhythm of mitoses in the corneal epithelium of the animals irradiated with a dose of 190 r did not differ appreciably from the corresponding curve of the control mice. The time of the maximal number of mitoses in the experimental animals coincided with that in the controls (8 A.M.). The slight difference between the numbers of mitoses in the corneal epithelium of the two groups of animals was not statistically significant ($P = 0.44$).

The mitotic activity of the experimental animals reached a minimum during the evening (8 P.M.). The difference between the maximal and minimal numbers of mitoses in both the control and the experimental animals was highly significant (for the control group $P = 0.001$ and for the experimental group $P = 0.002$).

Diurnal Changes in Mitotic Activity in the Corneal Epithelium of Mice after Whole-Body Irradiation (mean values obtained on 5 animals are given for each group)

Dose of irradiation (in r)	Time of sacrifice of animals (hour of day)	Mitotic index					
		peripheral zone of cornea		central zone of cornea		cornea as a whole	
		control	experiment	control	experiment	control	experiment
190	8	395±26	333±34	348±45	316±36	379±29	337±34
	14	96±13	98±18	159±19	108±8	119±14	102±14
	20	74±10	96±26	47±7	26±6	64±7	71±19
	2	167±25	299±15	82±25	153±25	133±21	240±10
	8	373±17	337±15	413±28	315±37	407±17	332±22
Mean daily number of mitoses		183	206	159	151	174	188
400	8	274±24	312±26	383±28	294±8	314±24	305±17
	14	98±7	182±26	193±12	196±19	139±9	186±22
	20	121±25	166±18	126±35	81±18	122±25	132±16
	2	204±29	236±19	97±20	118±20	162±26	192±15
	8	344±30	284±9	292±37	259±8	321±29	273±9
Mean daily number of mitoses		174	224	200	170	184	204

It will be seen from the table that the mitotic activity of the corneal epithelium of the control and experimental mice failed to coincide only at night (at 2 A.M.). In this case the number of mitotic divisions in the corneal epithelial cells of the experimental animals exceeded the mitotic index of the control group. The observed difference was statistically significant ($P = 0.003$). It must be pointed out, however, that the number of mitoses in the irradiated mice at the beginning of the experiment (at 8 A.M.) and at its end (8 A.M. on the second day) was the same (337 and 332 mitoses in 100 fields of vision). Furthermore, the sweep of the diurnal variations in the number of mitoses in the corneal epithelium of the experimental mice coincided roughly with the control range (in the latter six-fold, in the former five-fold). No significant difference could be observed, likewise, in the mean daily number of divisions. In the control animals the mean daily mitotic index was 174 mitoses in 100 fields of vision, and in the experimental animals 188 mitoses.

Hence, the diurnal rhythm of mitotic activity of the corneal epithelium of mice irradiated with a dose of 190 r when investigated on the 5th day after irradiation, was very similar to the diurnal rhythm in the control mice.

Second series of experiments. The table shows that the diurnal rhythm of mitosis in the corneal epithelium of the mice irradiated with a dose of 400 r was also preserved. The diurnal fluctuations were marked by a maximal number of mitoses during the morning (8 A.M.) and a minimal number during the evening (8 P.M.). The difference between the maximal number of mitoses in the experimental and control animals was highly significant ($P = 0.002$). An increase in the intensity of mitotic activity in the corneal epithelium of the experimental mice took place at 2 P. M. and 2 A. M., but in both cases the difference between the number of mitoses in the experimental and control animals was not statistically significant (for 2 P.M., $P = 0.12$, and for 2 A.M., $P = 0.50$). Moreover, the maximal number of mitoses at the beginning of the experiment (8 A.M.) and at its end (8 A.M. next day) remained at approximately the same level.

The range of the diurnal variations in the number of mitoses in the corneal epithelium of the experimental animals was two-fold, and that in the control animals three-fold. The mean daily mitotic activity in the experimental animals showed a tendency toward an increase. In the control mice, for instance, the mean daily number of cell divisions was 184 mitoses in 100 fields of vision, and in the experimental animals 204 mitoses.

The results thus show that on the 5th day after irradiation of mice with a dose of 400 r, while the intensity of the mean daily mitotic activity was very slightly increased, the diurnal periodicity of the mitoses in the corneal epithelium of the mice was preserved.

We also analyzed the findings relating to the topographical distribution of mitoses in different zones of the corneal epithelium of the mice after irradiation with doses of 190 and 400 r (see table). In the experimental animals (first and second series of experiments) in contrast to the controls, at 2 P.M. the numbers of mitoses in the peripheral and central zones of the cornea were roughly identical. At 2 A.M. the mitotic activity was increased at the periphery of the cornea in both experimental and control mice.

Finally, when we compared the relative percentages of the phases of mitosis in the corneal epithelium of the irradiated and control mice, we could detect no changes. So far as chromosomal disturbances in the corneal epithelial cells undergoing mitotic division are concerned, in the mice exposed to irradiation in a dose of 400 r pathological forms of mitotically dividing cells were observed (giant mitoses, bridges, etc.).

It should be noted that the different doses of radiation (190 and 400 r), which cause cell damage [5, 6] and also radiation sickness [4], did not lead to any appreciable disturbances of the diurnal periodicity of cell proliferation in the corneal epithelium of the mice. It has been reported [1] that the diurnal periodicity of the mitotic activity in the corneal epithelium of mice is disturbed after exposure to high doses of radiation (750 r). A decrease in mitotic activity was observed at 10 A.M. and an increase in the number of mitoses at 1 P.M. We also observed a similar increase in the intensity of cell division in the corneal epithelium of mice at 2 P.M., although the diurnal rhythm of mitotic activity on the whole was preserved.

Our results thus demonstrate that, despite slight variations in the number of mitoses at certain times of day or night, in general the diurnal rhythm of mitosis was not disturbed after irradiation of mice in doses of 190 and 400 r. It may accordingly be concluded that these doses of radiation have no appreciable effect on the mechanisms responsible for the diurnal periodicity of cell reproduction.

SUMMARY

A study was made of the effect produced by ionizing radiation (190 and 400 r) on the 24-hour periodicity of mitotic activity of the mouse corneal epithelium. As noted, the 24-hour mitotic rhythm was retained on the 5th postirradiation day (doses of 190 and 400 r being used). The time of the maximal (8 A.M.) and the minimal (8 P.M.) number of mitosis in the corneal epithelium of experimental mice coincided with control indices. The difference between the maximal and minimal mitotic activity in the two experiments was highly significant. The range of the 24-hour variations of the number of mitoses showed no significant change in the experimental animals. After irradiation of mice in a dose of 400 r there was a tendency to a rise of the mean 24-hour intensity of cellular multiplication.

LITERATURE CITED

1. G. P. Gruzdev, Radiobiologiya 1, 1, 52 (1961).
2. L. P. Kosichenko, Byull. éksper. biol. i med., 6, 98 (1960).
3. L. P. Kosichenko, Doklady Akad. Nauk SSSR 138, 4, 982 (1961).
4. N. I. Nuzhdin, N. I. Shapiro, and O. N. Petrova, Collected Papers on Radiobiology [in Russian], p. 83, Moscow, 1955.
5. N. I. Nuzhdin and O. P. Dogmareva, Doklady Akad. Nauk SSSR 125, 3, 650 (1959).
6. I. M. Shapiro, Doklady Akad. Nauk SSSR 114, 4, 760 (1957).
7. F. Halberg, C. Barnum, R. Silber, and J. Bittner, Proc. Soc. exp. Biol. and Med., 1958, v. 97, No. 4, p. 897.
8. R. Vasama and R. Vasama, Acta Anat., 1958, v. 33, No. 3, p. 230.
