PROLIFERATIVE ACTIVITY OF ŠVEC'S TUMOR AFTER SINGLE AND REPEATED IRRADIATION

S. L. Potapov and L. V. Sokolova

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Autoradiographic investigations of an experimental tumor after local irradiation in a dose of 600 rad showed no delay in passage of the cells through the cycle at the stages of 6 and 12 h. A marked decrease in mitotic index (MI) and index of labeled nuclei (ILN) was observed 96 h after irradiation. Reirradiation in a dose of 1200 rad after an interval of 18 h led to a marked decrease in MI and ILN through blocking the transition from the G to the S period. By 24-48 h, however, cell proliferation was restored. The high MI found at all times of the investigation after repeated irradiation of the tumor with an interval of 24 h was probably due to an increase in the actual duration of mitosis, as is confirmed by the noticeable increase in the number of the late phases of mitosis.

KEY WORDS: DNA synthesis; mitotic activity; irradiation.

Facts now available show that, when the sessional dose and interval between sessions of irradiation are chosen, the morphological structure of the tumor tissue and the character of the mitotic cycle of the tumor must be taken into consideration [1, 4-7].

This makes it important to study the response of a tumor to irradiation at times of maximal mitotic activity and when the largest number of cells are in the phase of synthesis.

EXPERIMENTAL METHOD

Experiments were carried out on 119 male Wistar rats weighing (average) 230 g with a transplanted Švec's tumor. This strain of tumor was obtained in 1957 by injecting the filtrate of a mesenchymal-epithelial BS tumor into splenectomized young Wistar rats. Švec's tumor is nowadays regarded as a reticulosis–hemocytoblastosis [2]. The response of the tumor to a single local irradiation in a dose of 600 rad and repeated irradiation in a dose of 600 rad at intervals of 18 and 24 h was studied in order to compare the beginning of regeneration in the tumor. The dose of 600 rad and the intervals of 18 and 24 h used in this series of experiments were chosen mainly on the basis of earlier autoradiographic data for sarcoma SSK of rats [3]. Even a relatively small difference in the dose for repeated irradiation or in the interval between sessions has been shown to result in great differences in the number of surviving cells [5]. The animals were killed and material taken for investigation 6, 12, 18, 24, 30, 48, and 96 h after the end of irradiation, for which purpose thymidine-H^3 was injected intraperitoneally, 4 h previously, in a dose of 0.5 μCi/g (specific activity 11.6 Ci/mmole). Irradiation was given by the LUÉ-5 linear accelerator with bremsstrahlung energy of 2.5 MeV at a dose rate of 80 rad/min, and with a source–surface distance of 100 cm. Autoradiographs were prepared by the usual method. The percentage of cells in the phase of DNA synthesis was determined from the index of labeled nuclei (ILN), the mitotic index (MI) by counting 1000 tumor cells, the number of grains of silver in 50 cells, and the number of labeled mitoses. The significance of differences between the mean results was determined by the Fisher–Student method.

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TABLE 1. Mitotic Activity and DNA Synthesis in Tumor after Single Irradiation in Dose of 600 rad (% of control)

Time after irradiation (in h)	MI	ILN	Mean No. of grains of silver per cell	Labeled mitoses
6	110,0	160,5	86,3	55,4
12	116,3	99,8	115,3	113,2
18	86,0	97,6	86,1	79,5
24	137,0	159,4	66,1	61,4
30	80,0	73,6	105,2	86,4
96	48,3	56,3	86,4	121,6

TABLE 2. Mitotic Activity and DNA Synthesis in Tumor after Irradiation in Dose of 1200 rad (2 · 600 rad) at Intervals of 18 and 24 h (in % of control)

Time after irradiation (in h)	MI		ILN		Mean number of grains of silver per cell		Labeled mitoses	
	18 h	24 h	18 h	24 h	18 h	24 h	18 h	24 h
6	92,1	177,1	60,5	154,4	53,6	114,5	17,6	67,1
12	52,3	106,3	25,8	78,5	64,1	102,1	33,1	65,0
18	67,8	152,9	29,0	68,9	50,4	60,7	33,3	40,2
24	105,0	197,3	32,4	87,2	40,0	42,1	19,2	28,9
48	107,7	191,2	43,3	109,2	47,0	74,8	37,5	59,7

TABLE 3. Number of Aberrant Mitoses at Different Times after Irradiation in a Dose of 1200 rad (2 · 600 rad, interval 18 h)

Time after irradiation (in h)	Number of aberrant mitoses (in %)
6	23,8±5,8
12	16,0±4,3
18	4,1±2,5
24	17,6±5,6
48	19,1±4,8
Control	1,1±0,7

EXPERIMENTAL RESULTS

The results of investigation of the proliferative activity of the Švec's tumor after single and repeated irradiation are given in Tables 1 and 2. Changes in ILN in the tumor followed the dynamics of the number of dividing cells. A statistically significant increase in ILN was observed 6 and 24 h after irradiation ($P < 0.05$); no delay in the passage of the cells through the cycle was found; the tumor cells underwent mitosis and maintained a relatively high level of proliferation. ILN decreased gradually until 96 h.

The low values of MI in the tumor after 96 h were thus due to inhibition of DNA synthesis, for the rate of its renewal was 7% per diem. No signs of restoration of cell proliferation could be observed, whereas after irradiation of rat sarcoma SSK in an equivalent dose regeneration was fluctuating in character [3]. The number of labeled mitoses 6 h after irradiation was 55.4%; it remained about the same after 24 h. By 96 h the number of labeled mitoses showed a marked increase and was higher than the control until the end of observation.

The intensity of incorporation of thymidine-H^3 was lowered during the 4 days of observation.

Reirradiation of the tumor was carried out 18 and 24 h after the initial session in a dose of 600 rad, when labeled cells and mitoses in the tumor were comparatively numerous.

Cells in the phase of DNA synthesis are known to be more sensitive to the action of ionizing radiation than cells in other phases of the mitotic cycle. Analysis of the autoradiographs after repeated irradiation at an interval of 18 h showed a marked decrease in MI during the first 18 h, but complete recovery was observed later, whereas after irradiation at an interval of 24 h the values of MI were above the control level. Blocking of the transition of the cells from the G_2 into the M period was not found and the tumor cells were able to pass through the mitotic cycle, although in the early stage (6 h) as many as 23.8% of aberrant mitoses were discovered (Table 3).

Between 24 and 48 h after irradiation, metaphases were predominant and telophases were very rare. Possibly no division took place in these cells later. The increase in the number of prophases starting from 12 h after irradiation and, in particular, after 24 h indicates an active process of recovery of cell proliferation.

After repeated irradiation with an interval of 18 h ILN remained low for 48 h, during which the intensity of DNA synthesis fluctuated at around 50% of the control level. After irradiation with an interval of 24 h, ILN returned to the control level by 48 h and the intensity of DNA synthesis increased to 74.8% 48 h after irradiation; this probably corresponded to lengthening of the synthetic period.

After two sessions of irradiation with an interval of 18 h, roughly half of the tumor cells were thus unable to enter the phase of synthesis as a result of blocking the transition from the G_1 to the S period. The high value of MI observed at all times of investigation after irradiation with an interval of 24 h could probably be the result of a marked increase in the duration of mitosis itself, as is also shown by the increase in the number of late phases of mitosis. The decrease in the number of labeled mitoses at times of fewest

dividing cells suggests partial delay of the tumor cells in the G_2 period and also a slower passage of the cells through the cycle.

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