EFFECT OF A COMBINATION OF LOCAL HYPERTHERMIA WITH IRRADIATION ON TUMOR TISSUE HYDROLASE ACTIVITY

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The successful use of hyperthermia in combination with irradiation to treat malignant tumors necessitates an experimental study of the conditions for their optimal combination and, in particular, the duration of hyperthermia, as well as a study of the mechanisms of the radiosensitizing action of hyperthermia [1]. It has been suggested [9, 11] that lysosomal hydrolases play an important role in the mechanism of the effects of hyperthermia.

The aim of this investigation was to study changes in activity of lysosomal enzymes of tumor tissue such as acid phosphatase (AP) and cathepsin D (CD) after local exposure to ultra-high-frequency hyperthermia (UHF-hyperthermia) and x-ray irradiation.

EXPERIMENTAL METHOD

Experiments were carried out on 180 noninbred male rats weighing 200 g. A solid tumor (sarcoma 45) was transplanted subcutaneously into the right thigh. Hyperthermia was produced by means of the Luch-58 microwave apparatus (2450 MHz). The temperature detector, a thermoresistor, mounted on a needle, was introduced into the center of the tumor. Temperature was recorded while the UHF apparatus was temporarily switched off. The temperature in the tumor was maintained at 42.0 ± 0.5°C for 15 and 30 min or 60 min. The source of x-rays was a "RUM-17" apparatus (200 kV, 10 mA, dose rate 0.87 Gy/min). The dose range used in the investigation was 2-8 Gy. Both hyperthermia and x-ray irradiation were confined to the animal's limb with the tumor. The interval between application of heat and x-rays was 2-3 min.

Total and free AP activity was determined in tumor tissue homogenate relative to *p*-nitrophenyl phosphate [4] and CD activity was determined as hemoglobin [2]. To determine enzyme activity a weighed sample of tumor tissue was homogenized for 60 sec in 0.2% Triton X-100, and the milder conditions of homogenization (30 sec in 0.4% sucrose) enabled free enzyme activity to be determined in the supernatant. The coefficient of liberation of lysosomal hydrolases was determined as the ratio of free to bound enzyme activity. In turn, bound activity was obtained as the difference between total and free activity. Significance of differences between groups was determined by Student's t test.

EXPERIMENTAL RESULTS

When exposure to the two factors was given separately, x-ray irradiation in a dose of 2 Gy caused no appreciable change in total AP activity of the tumor tissue but reduced the free AP activity a little immediately after irradiation. Increasing the dose of irradiation to 4 and 8 Gy likewise caused a decrease in total AP activity, and by a greater degree than free activity, for the coefficient of hydrolase liberation exceeded 1 (Fig. 1).

UHF-hyperthermia for 15 min, unlike x-ray irradiation, significantly increased free AP activity and also increased total AP activity, although by a lesser degree. Increasing the duration of hyperthermia to 30 min or to 60 min lowered free AP activity without changing total activity.

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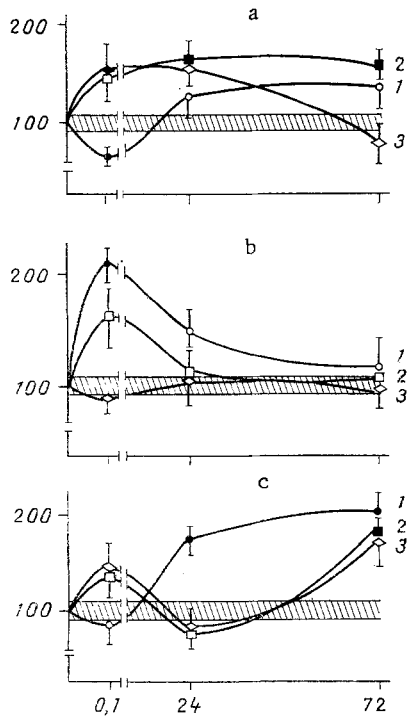

Fig. 1. Changes in ratio of free AP activity to bound activity at different times after combined and separate exposure to x-rays and UHF-hyperthermia. Abscissa, time of investigation after exposure (in h); ordinate, ratio of free to bound activity (in % of control). Shaded zone — changes in parameter in control. a) X-ray irradiation in doses of 2 Gy (1), 4 Gy (2), and 8 Gy (3); b) UHF-hyperthermia after exposure of 15 min (1), 30 min (2), and 60 min (3); c) combined exposure to both factors: 15 min + 8 Gy (1), 30 min + 8 Gy (2), 60 min + 8 Gy (3).

TABLE 1. Effect of UHF-Hyperthermia and X-Rays on Change (in %) in Total and Free CD Activity (M ± m)

Time of treatment (UHF-hyperthermia + irradiation)	CD activity	Time of investigation, h			
		before exposure	0,1	24	72
15 min + +2 Gy	Total	100±4	124±7*	108±5	86±7
	Free	100±3	110±6	94±12	72±6*
30 min + +4 Gy	Total	100±3	110±7	119±11	102±2
	Free	100±3	60±10*	100±12	83±7*
15 min + +8 Gy	Total	100±4	93±7	102±2	126±3*
	Free	100±2	89±10	94±12	150±10*
30 min + +8 Gy	Total	100±3	75±5*	88±2*	100±13
	Free	100±5	89±5	88±6	102±8
60 min +8 Gy	Total	100±4	86±7	85±5*	77±6*
	Free	100±4	84±10	88±4	100±89

Legend. *P < 0.05 compared with control (untreated).

Combined exposure to UHF-hyperthermia for 15 min and x-ray irradiation in a dose of 2 Gy led to maximal elevation of free activity compared with the same parameter after exposure to the two factors separately, evidently due to the release of enzymes from lysosomes, for total AP activity in this case was the same as in the group without treatment. With an increase in the duration of hyperthermia to 60 min and in the dose of x-rays to 8 Gy, total and free hydrolase activity was indistinguishable from its value in the control. The coefficient of liberation of enzymes from lysosomes 24 h after hyperthermia for 15 min and exposure to radiation in a dose of 8 Gy was higher than after hyperthermia for 30 min combined with the same dose of irradiation (Fig. 1). The fact will be noted that the time course of activity was practically identical in groups receiving combined exposure to UVH-hyperthermia for 30 min and x-rays in a dose of 8 Gy and those exposed to hyperthermia for 60 min and the same dose of irradiation.

Changes in total and free activity of another lysosomal enzyme, namely CD, are given in Table 1. They were independent of the duration of exposure to hyperthermia when its action was combined with that of x-rays.

It follows from these results that, first, with an increase in the dose of x-rays, liberation of lysosomal hydrolases into the intracellular medium increased, but by the 3rd day this parameter was substantially lower after irradiation in a dose of 8 Gy, whereas after irradiation in doses of 2 and 4 Gy it remained higher than in the control ($P < 0.05$); second, with an increase in the duration of exposure to heat from 15 to 30 or to 60 min, liberation of enzymes from lysosomes decreased; this parameter, moreover, also decreased with an increase in the time after exposure until 3 days; third, in the case of combined exposure to UHF-hyperthermia and x-rays the duration of exposure to heat had no significant effect on liberation of hydrolases.

In experiments on sarcoma-45, immediately after the end of hyperthermia maximal liberation of hydrolases was observed after exposure of 15 min compared with that after exposures of 30 and 60 min, whereas in experiments with HeLa cells [10] AP activity was shown to be a linear function of duration of heating (42-44°C, exposure from 0 to 90 min). Meanwhile, the data correlate with others [13] obtained after heating of a Novikoff's hepatoma (43°C, 30 and 60 min). The authors cited showed a very small change in liberation of lysosomal enzymes with an increase in the duration of hyperthermia from 30 to 60 min (80 and 84%, respectively, for AP). Considering the function of lysosomes (participation in degradation processes and elimination of injured cell structures) and the direct correlation [8] between thermal inactivation of cells and increased activity of lysosomal hydrolases, and also Ardenne's hypothesis [5] of the chain action of enzymes liberated from lysosomes, it can be tentatively suggested that increased activity of lysosomal hydrolases in the intracellular medium on account of labilization of the lysosomal membranes is important for the radiosensitizing effect. We know [7] that lysosomal membranes are one of the targets in heat damage. The writers showed previously [3] that an increase in the duration of exposure to heat from 15 to 30 min, if combined with x-ray irradiation, does not affect the time course of growth of sarcoma 45. There is evidence [12] that the combined action of x-rays in a dose of 8 Gy and hyperthermia at 42°C delays growth of cartilage in young rats; it has been shown, moreover, that an increase in the duration of heating from 15 to 40 min in the case of combined exposure was not reflected in the degree of damage.

The facts described above thus indicate the important role of liberation of lysosomal hydrolases in mechanisms of the radiosensitizing action of hyperthermia. The maximal effect was achieved as a result of 15-min exposure to hyperthermia. An increase in the duration of heating made lysosomal hydrolases less effective and did not increase the antitumor effectiveness of combined exposure. Determination of lysosomal hydrolases can be used in the choice of optimal conditions for antitumor therapy by a combination of hyperthermia and radiation.

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