Effects of the Sequential Action of Heat and Radiation on Yeast Cells

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1. Introduction

Hyperthermia combined with ionizing radiation is widely used in hyperthermic oncology [1]. Recently it has been shown for heat combined with either ionizing radiation [2] or UV light [3] that the recovery constant defining the probability of recovery was independent of thermal load, while the portion of irreversibly damaged cells gradually increased as a function of heat treatment duration after a sequential treatment of these agents and as a function of the exposure temperature after simultaneous action of these agents. In that context, the synergistic interaction of ionizing or UV radiations and hyperthermia is not related with the impairment of cell recovery capacity itself and that the observed decrease in the rate and the extent of recovery after combined action of these modalities may be attributed to the increased yield of irreversible damage. The present work was done to study experimentally the effectiveness of interaction of heat and ionizing radiation after their sequential treatment.

2. Materials and Methods

The wild-type diploid yeast *Saccharomyces cerevisiae*, strain XS800, was used in this study. Yeast cells were incubated for 5 days at 30 ºC on a complete nutrient agar layer before they were irradiated with γ-rays at 2 and 80 Gy/min. Hyperthermia was administrated in a water bath in which a desired temperature ± 0.1 ºC was maintained. After the treatments, a known number of cells were plated so that 150-200 colonies per dish would be formed by the surviving yeast cells after 5-7 days of incubation at 30 ºC. Each point was calculated from the colony numbers of triplicate dishes. All experimental series were repeated 3–5 times, and the mean survival values were calculated from at least three experiments.

3. Results and Discussions

Cells were subjected to ionizing radiation before or after heat exposure. Postheating and preheating cells markedly increased cell sensitivity to ionizing radiation (Fig. 1). The amount of radiosensitization was quantitatively estimated by the thermal enhancement ratio (TER), calculated as the ratio of slopes. The degree of radio-sensitization tends to increase with an increasing heat exposure.

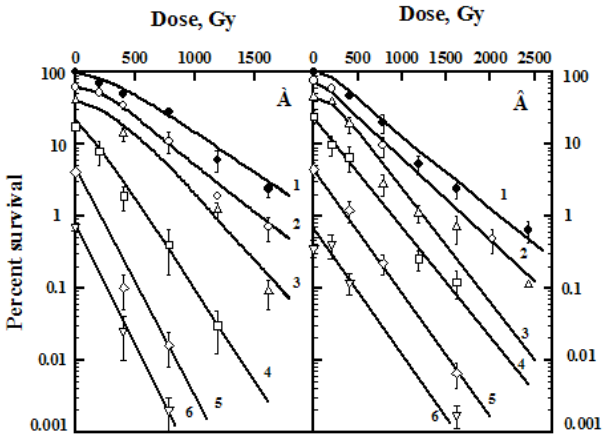

Fig. 1. Survival curves of yeast cells. A, sequential treatment with ionizing radiation (2 Gy/min) and heat (50 ºC); B, the reverse order of these agents. The duration of heat exposure, hr : 1- 0, 2- 0.5, 3-1.5, 4-3, 5- 6, 6- 9.

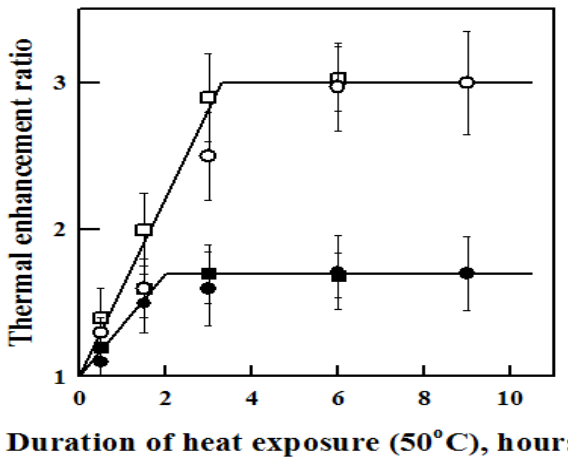

Fig. 2. The dependence of TER on the duration of heat exposure (50 ºC) after the sequential action of ionizing radiation and hyperthermia: irradiation (2 Gy/min)+50 ºC (open circles); the reverse order of these agents (closed circles);irradiation (80 Gy/min)+50 ºC (open squares); the reverse order of these agents (closed squares).

The dependence of the TER on the duration of heat exposure applied after (open circle) or before irradiation (closed circles) was shown in Fig. 2. The increase in the TER values shows a progressive increase in radiation sensitivity to increasing duration of heat exposure. It is also evident that in both cases there is a value of heat exposure duration (about 2-3 hours) after which no further increase in the cell radiosensitivity can be observed. Note that the sequence of the combination is very important. Ionizing radiation applied before heating was more effective than irradiation after heating, i.e. a maximum of TER of 3.0 vs. 1.7.

From a set of survival curves for yeast cells irradiated with γ-rays (2 Gy/min) alone or combined with heat exposure (58 ºC), it becomes evident that cells treated for 0.5 and 1.0 min after ionizing radiation induced a small amount of radioresistance. These results suggest that a certain level of cytotoxicity from hyperthermia alone is required to achieve a radio-enhanced cell killing after combination of ionizing radiation at 2.0 Gy/min and acute hyperthermia (58 ºC). The amount of the induced radiosensitization and radio-resistance was estimated by TER.

Fig. 3 shows the dependence of TER on the duration of heat exposure. The limit values of the TER were 1.5 independently of the sequence of combination.

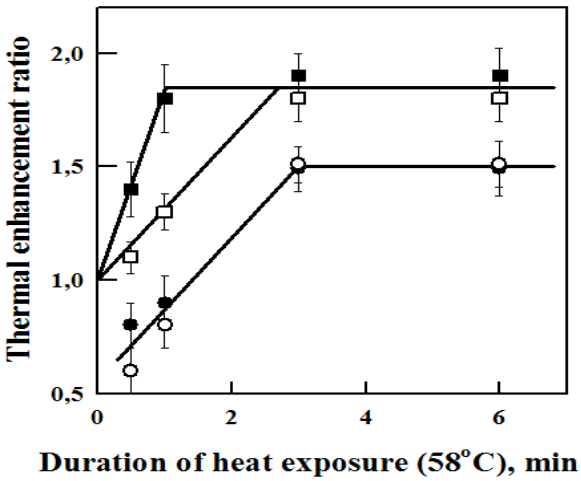

Fig. 3. The dependence of TER on the duration of heat exposure (58 ºC) after the sequential action of ionizing radiation and hyperthermia on diploid yeast cells: irradiation, 2 Gy/min + 58 ºC (open circles); the reverse order of these agents (closed circles); irradiation, 80 Gy/min + 58 ºC (open squares); the reverse order of these agents (closed squares).

The present studies provide information on the dependence of the thermal enhancement ratio after the sequential action of heat and ionizing radiation on the dose and dose rate of ionizing radiation as well as on the temperature and duration of heating.

It is worth noting that our data for yeast cells support studies with mammalian cells [4]. Specifically, for high temperature where there is more killing from heat alone than for lower temperature where there is less killing from heat alone, cell killing for heat combined with ionizing radiation is independent of the order of treatment, i.e. heat before irradiation give the same killing as heat after irradiation. Our data make it clear that for the lower temperature the sequence γ-ray + heat give more cell killing than the inverse order of treatment, with the amount of cell killing for heat after exposure to ionizing radiation being greater for 50 ºC than for 58 ºC.

4. Conclusions

It was concluded in this paper that, when the lethal effect from hyperthermia alone is minimal, lethality from combined action of ionizing radiation and thermal treatment is greater when hyperthermia follows irradiation than when hyperthermia precedes irradiation. It indicates the general responses of yeast and mammalian cells to the combined action of heat and ionizing radiation. The results of this study demonstrate the possibility of a reconcilable explanation of experimental data on the sequential action of heat and ionizing radiation with yeast cells.

ACKNOWLEDGEMENTS

This work has been carried out under the National R&D Program by the Ministry of Education, Science and Technology (MEST) of Korea. It has also been supported by International Cooperation, and by Russian Funds of Fundamental Researches, Humanity Sciences and Administration of Kaluga Region (grant No. 08-06-59609).

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