Exposure Rate as a Determinant of the Synergistic Interaction of Heat Combined with Ionizing or Ultraviolet Radiation in Cell Killing

JIN KYU KIM1*, VLADISLAV G. PETIN2 and GALINA P. ZHURAKOVSKAYA2

1Korea Atomic Energy Research Institute, 150 Dukjin-dong, Yusong-gu, Taegon, 305–353 Korea
2Medical Radiological Research Center, Koroleva str. 4, Obninsk, Kaluga Region, 249036 Russian Federation

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A synergistic interaction of hyperthermia with ionizing or UV light (254 nm) radiation was analyzed in experiments with yeast cells. For a fixed dose rate of radiation, the synergism was shown to be observed only within a definite temperature range (40–45°C), inside of which there was an optimal temperature to achieve the highest synergism. The effectiveness of the synergistic interaction was smaller for haploid cells than for diploid cells. Experimental data from diploid yeast cells evidenced the significance of the exposure rate in the synergistic interaction of heat combined with ionizing or ultraviolet radiation. The data show that the less is the intensity of radiation, the lower is the temperature that should be used to provide some definite, or highest, synergistic interaction with the radiation. To demonstrate the significance of this rule for other cellular systems, the results of other authors published for bacterial spores and mammalian cells are discussed. Calculations from these results have confirmed the revealed relationship between the dose rate and the exposure temperature. On this basis, it is inferred that synergism may take place at small intensities of harmful environmental factors existing in the biosphere. Hence, any assessment of the health or environmental risks should take into account synergistic interactions between harmful agents.

INTRODUCTION

The exposure rate constitutes a significant parameter in radiation research. While it is generally accepted that lowering the dose rate leads to a decrease in the cell-killing efficiency per unit dose1,2), the matter is less clear for the synergistic interaction of agents combined together3,4). At the same time, combined exposures are an essential feature of modern life. It is
well known that almost all physical, and a wide array of chemical, harmful agents, both natural and man made, are capable of interacting with each other in a synergistic manner when the combined biological effect exceeds the results of individual effects produced by interacting agents. Therefore, any risk assessment must consider the question of whether combined effects will influence the health outcome. Of all possible situations of combined action, the long-term exposure of living objects to low levels of the agents widely presented in nature is especially important\(^5,6\). However, an assessment of the potential significance of synergistic interactions between adverse environmental factors acting together at the level of intensity and concentration found in the biosphere is still an intriguing and unresolved problem. Real experiments with low intensities found in environmental and occupational settings is prone to large uncertainties. A feasible approach to this problem is to analyze the dependence of the efficiency of the synergistic interaction on the intensity of the agents used. Hypothetically, some possibilities could be realized. The case when synergism is decreasing with the intensities of applied deleterious agents is unimportant. The same is true for the situation when a decrease in the intensity of one factor should be accompanied by an increase in the intensity of another to retain their synergistic interaction at the same level. The only possibility would be of great importance: if the lesser intensity of one of the agents is applied, then the smaller intensity of another agent should be employed to display the highest, or a definite, level of synergy. In such a case, it would be expected that environmental agents, even at low intensity, may, in principle, interact with each other in a synergistic manner, and thereby enhance their harmful action. In this paper, we present conclusive evidence confirming the last opportunity for various cellular systems, thus demonstrating that the exposure rate is a determinant of the synergistic interaction of heat combined with ionizing or ultraviolet radiation in cell killing. Some results have already been published concerning the simultaneous action of heat and ionizing radiation\(^7,8\), or UV light\(^9,10\) on yeast cells. Here, we present more complete results while emphasizing the dependence of synergy on the exposure rate of the applied agents. To display the applicability of the regularities obtained with yeast cells for other cellular systems, we present here the results of our calculations based on the papers of other authors concerning the simultaneous action of hyperthermia and ionizing radiation on bacterial spores\(^11,12\) and mammalian cultured cells\(^13,14\). Thereby, the importance of the exposure rate for synergistic interactions between heat and other inactivating agents on cellular systems differing in biological complexity and their sensitivity to various physical agents will be demonstrated.

**MATERIALS AND METHODS**

*Zygosaccharomyces bailii* haploid as well as *Saccharomyces cerevisiae* diploid (strain XS800) and haploid (strain S288C) yeast cells were used in the experiments. Yeast cells were incubated before irradiation for 3–5 days at 30°C on a complete nutrient agar layer to a stationary phase. Aliquots with 10\(^6\) yeast cells/ml were exposed to ionizing or UV light radiation applied alone or combined with hyperthermia. We used a \(^{60}\)Co γ-ray source (Gammacell 220, Atomic Energy of Canada LTD). The γ-ray dose-rate, estimated by a Siemens ionization
chamber, was 11.8 Gy/min. To provide an opportunity to vary the dose rate of ionizing radiation within a wide range, we used an electron beam from a 25-MeV pulsed linear accelerator. The pulse duration at half-peak output was 2.7 µs, and the pulse repetition rate was at 50 Hz; the average dose rates were 5, 10, 25 and 250 Gy/min, as determined by ferrous sulphate dosimetry.

For ultraviolet (UV) irradiation, the cells were exposed with a germicidal lamp that emitted predominantly UV light at a wavelength of 254 nm. A variation of the intensity was achieved by means of calibrated metal wire nets. The fluence rate was measured using a calibrated General Electric germicidal meter. To avoid any photoreactivation, UV exposure, dilution and cell plating were performed under red ambient light, while post-irradiation incubation was carried out under dark conditions.

Experiments on the simultaneous action of high temperature with ionizing or UV light radiations were performed as follows. A special dish and tube containing 1.4 (for UV light irradiation) or 9.9 (for ionizing radiation) ml of sterile water was preheated to a specified temperature, which was maintained with an accuracy of ±0.2°C. A cell suspension was added immediately before irradiation (an 0.1-ml aliquot containing 1.5×10⁶ cells for UV-light and 10⁷ cells for ionizing radiation exposure). For the simultaneous action of hyperthermia and radiation, the time interval between introducing the cells into the preheated water and the beginning of exposure was about 0.1–0.3 min, which was significantly less than the total treatment time. At the end of the treatment, the samples were rapidly cooled to room temperature and, hence, the exposure to high temperature alone or combined with either ionizing or UV light radiation lasted for the same period of time. Thereafter, the cell suspension was diluted to the necessary concentration and plated onto the standard nutrient medium to determine the cell survival by the macrocolonies method. All experimental series were repeated 3–5 times. The details have been described elsewhere⁷–¹⁰).

RESULTS

It is now generally accepted that the highest synergistic interaction is observed under the simultaneous action of harmful agents. The increase during the interval between exposures results in a diminution of synergy. That is why in the present study we analysed the simultaneous application of agents. To determine whether the interactions of hyperthermia and radiation treatments are additive or synergistic, data are usually calculated according to a definition recommended by the international commission on radiation units¹⁶), and have since been used by many authors⁷,⁸,¹⁷): an independent interaction (additivity) is determined by $SF_C = SF(HT) \times SF(RT)$ and synergy is observed if $SF_C < SF(HT) \times SF(RT)$, where $SF(HT)$ and $SF(RT)$ stand for the surviving fractions after treatments with hyperthermia and radiation applied alone, respectively. $SF_C$ stands for the surviving fraction after the simultaneous treatment of both modalities.

Fig. 1 provides an example of the basic experimental data used in this investigation. It is appropriate to mention here again that both agents act simultaneously, i.e. the exposure time
was identical for both high temperature and radiation; thus, the upper scale for the exposure time concerns all of the curves presented. Four types of survival curves were obtained for every condition of thermoradiation action: a heat treatment alone (curve 1), ionizing radiation without heating (curve 2), composite simultaneous heat and radiation exposure (curve 4). Curve 3 represents a theoretically expected survival curve which would be obtained if inactivation by composite heat and radiation were completely independent, i.e. it was calculated according to $SF_C = SF(HT) \times SF(RT)$. To quantitatively estimate the sensitization action of hyperthermia, one can apply the thermal enhancement ratio$^{15}$, defined as $D_3/D_1$ (Fig. 1). This ratio indicates an increase in cell radiosensitivity by high temperature. However, it does not reflect the kind of interaction (whether it was independent or synergistic). To quantitatively evaluate the synergistic effect we used the synergistic enhancement ratio$^7$, defined as $D_2/D_1$ (Fig. 1). For exponential survival curves, this parameter is independent of the survival level for which it is calculated. As soon as the exposure time of both agents became identical, there was no problem to estimate a theoretical curve according to $SF_C = SF(HT) \times SF(RT)$, presented above. An uncertainty, including the envelope of additivity$^{16}$, can arise mainly for the consecutive application of agents when their doses are changed independently of each other. This wasn’t the case in the present study. However, in some of our experiments, sigmoidal

**Fig. 1.** Survival curves of *Zygosaccharomyces bailii* haploid yeast cells: curve 1 – heat treatment (45°C) alone; curve 2 – ionizing radiation (60Co) at 11.8 Gy/min and room temperature; curve 3 – calculated curve for independent action of ionizing radiation and heat; curve 4 – experimental curve after simultaneous thermoradiation action.
after ionizing or UV light survival cures might be transformed at a high exposure temperature into exponential ones\(^7,18\). For such a case, both the thermal and synergistic enhancement ratios were slightly dependent on the level for which they were calculated. That is why for sigmoidal survival curves, both of these parameters were calculated here for 10% survival. It is worth noting that in this study 78 sets of survival curves (Figure 1) were employed under different schedules of combined actions, including 48 sets for yeast cells, 16 for bacterial spores and 14 for mammalian cells. They are not presented here, but were used to calculate the dependence of the synergy on both the exposure temperature and the intensity of the applied radiation.

The thermal enhancement ratio (curves 1) and the synergistic enhancement ratio (curves 2) are plotted in Fig. 2 against the irradiation temperature for XS800 diploid (Fig. 2a) and S288C haploid (Fig. 2b). A noticeable feature of Fig. 2 is that the thermal enhancement ratio (curves 1) increases indefinitely with increasing exposure temperature, while the synergistic enhancement ratio (curves 2) at first increases, then reaches a maximum, which is followed by a decrease. This implies that a synergistic interaction between hyperthermia and ionizing radiation is observed only within a certain temperature range. Noteworthy is the fact that such a dependence of the synergistic effect on temperature under which the exposure occurred was also obtained upon the simultaneous combination of hyperthermia with UV light\(^9,10\), ultrasound\(^19\) and some chemical inactivating agents\(^20\). Hence, one can conclude that for a given intensity of physical factors or concentration of chemical agents there would be a specific temperature that maximizes the synergistic interaction. Any deviation of temperature from the optimal value results in a decrease of synergism. One can also see from these findings that the effectiveness of the synergistic interaction was smaller for haploid cells than for diploid cells.

To evidence the importance of synergistic effects at low intensity of inactivating agents, we analysed the dependence of the synergistic interaction on the dose rate of the ionizing or ultraviolet radiation applied in combination with hyperthermia. Using survival curves for dip-

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**Fig. 2.** Thermal enhancement ratio (\(\beta\), curves 1) and synergistic enhancement ratio (\(\delta\), curves 2) of *Saccharomyces cerevisiae* diploid (a, strain XS800) and haploid (b, strain S288C) cells as a function of the temperature applied (\(^{60}\text{Co}\)) at 11.8 Gy/min.
loid yeast cells after the simultaneous action of hyperthermia with ionizing radiation or UV light, we calculated the synergistic enhancement ratio for various irradiation conditions. This allowed us to establish a correlation between the dose rate of the ionizing radiation (Fig. 3a) or the fluence rate of ultraviolet light (Fig. 3b) and the exposure temperature, which both provide the highest synergistic interaction. The open circles denote the complete results of our calculations based on all of the experimental results obtained here and published earlier for ionizing\textsuperscript{7,8)} and UV\textsuperscript{9,10)} radiation combined with heat. One can see from Fig. 3a,b that direct proportional relationships exist between the exposure temperature and the intensity of radiation – the lesser the intensity of radiations applied, the smaller exposure temperature should be used to provide the highest synergistic interaction, and \textit{vice versa}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Correlation of the dose rate of ionizing radiation (a, c, d) or the ultraviolet light fluence rate (b) and the applied temperature providing the same synergistic interaction under simultaneous thermoradiation action: a, b – diploid yeast cells (\textit{Saccharomyces cerevisiae}, XS800); c – bacterial spores (\textit{Bacillus subtilis}); d – cultured mammalian cells (Chinese hamster cells). The data for yeast cells (a) were obtained by using a 25-MeV electron unit for irradiation. The original survival curves data were partly obtained by the authors for yeast cells, and were taken from the following references: 7, 8 for a, 9, 10 for b, 11, 12 for c and 13, 14 for d. The calculated values of synergism were 2.2 for bacterial spores, 1.7 for yeast cells, and 2.2 for cultured mammalian cells. The lines were drawn by eye.}
\end{figure}
To demonstrate the significance of this rule for other cellular systems, the results of other authors published for bacterial spores\textsuperscript{11,12} and mammalian cultured cells\textsuperscript{13,14} were involved. Our calculations from these results revealed the relationship between the dose rate and the exposed temperature observed for yeast cells. Figs. 3c and 3d show the correlation between the dose rate of ionizing radiation and the exposure temperature providing the same level (k = 2.2) of synergistic interaction of simultaneous action of heat and ionizing radiation for bacterial spores (Fig. 3c) and mammalian cells (Fig. 3d). The value of synergism was the highest for bacterial spores (k\textsubscript{max} = 2.2) and was intermediate for cultured mammalian cells (k = 2.2). This was due to the fact that for a mammalian cell system the highest synergistic effect was not obtained for all dose rates used in the experiments\textsuperscript{13,14}. These data, like for yeast cells, show the existence of a linear relationship between the dose rate employed and the temperature at which irradiation occurred: if the dose rate is decreased the exposed temperature should also be lowered to achieve the same level of synergistic interaction.

DISCUSSION

In this study, the synergistic interaction of hyperthermia applied simultaneously with ionizing radiation or UV light (254 nm) was analyzed in experiments with yeast cells. It was shown that the effectiveness of the synergistic interaction was smaller for haploid cells than for diploid cells. This is in agreement with the well-known fact that the mechanism of synergy is related to a cell’s ability to repair radiation damage\textsuperscript{13,14}, while the repair of DNA double-strand breaks requires two homologous DNA duplexes\textsuperscript{21}.

The second interesting fact obtained here is that for a constant intensity of radiation there was a specific temperature at which the synergistic interaction showed the highest effectiveness. In other words, there exists a definite temperature range inside which a synergistic interaction takes place. Similar data were observed for other inactivating or mutagenic agents: ultrasound and hyperthermia\textsuperscript{19}, chemical agent (cisplatin) and hyperthermia\textsuperscript{20}, chemical mutagen (1,2-dibromoethane) and X-rays\textsuperscript{22}.

It is well known that a synergistic interaction may be attributed to a reduced ability to repair radiation damage after the combined action of heat with either ionizing or UV light radiation\textsuperscript{13,14,23}. Our results with haploid and diploid yeast cells (Fig. 2) and the lack of synergy in different radiosensitive yeast strains defective in radiation repair\textsuperscript{7} are consistent with this hypothesis. Thus, little synergistic interaction at low temperatures means that the absorbed heat energy was too small to reduce the repair processes. The loss of synergy at relatively high temperatures can be ascribed either to the effect of saturation ("overkill effect") or a negligible lethal contribution of ionizing or UV light radiation applied together with hyperthermia. These results, besides being an important key for searching the synergy, can be considered as an indication of the possibility to optimize and achieve a desirable level of synergy. Some attempts to optimize thermoradiation action for cell inactivation have been undertaken\textsuperscript{3,8,11}.

The most remarkable feature, followed by the data presented in this paper, is the evi-
dence that the exposure rate is a determinant of the synergistic interaction of heat combined with ionizing or ultraviolet radiation in cell killing. It was demonstrated here for various cellular systems that the intensity of both forms of radiation is crucial for the synergy effect to be displayed – for a lower intensity, a relatively lower temperature must be used to provide the highest synergy, and vice versa. Therefore, for a short duration of interaction, relatively higher intensities and higher temperatures should be used to provide the greatest synergistic interaction. In contrast, for a long duration, relatively lower intensities and lower temperatures must be used. These results seem to be attributed to the fact that, if the intensity decreases, the effective lethal dose is delivered over a long time, so that the duration of heat incubation increases, which could explain the lower temperature that should be applied to the cells.

Despite the fact that all of the data presented were obtained at temperatures far beyond the physiological range, it is not excluded, in principle, that there could be optimal intensities of harmful agents existing in the biosphere and capable of interacting with the physiological heat of animals and man in a synergistic manner. Thus, it can be concluded that for a long duration of interaction, which is important for problems of radiation protection and health physics, low intensities of deleterious environmental factors may synergistically interact with each other either with environmental heat or physiological temperatures of homiothermal animals and men. Hence, the assessment of health or environmental risks from numerous natural and man-made agents at the level of intensities or concentrations found in environmental and occupational settings should, in principle, take into account any synergistic interaction between harmful agents.

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