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Radiosensitivity, liquid-holding recovery and relative biological effectiveness of densely-ionizing radiation after repeated irradiation of yeast cells

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ABSTRACT

Experimental results described earlier showed significantly larger relative biological effectiveness (RBE) values for wild-type diploid cells in comparison with radiosensitive mutants. This aspect was further studied in this paper. Diploid yeast cells were irradiated with gamma rays from ^{60}Co and alpha particles from ^{239}Pu in the stationary phase of cell growth. Survival curves and the kinetics of the liquid-holding recovery were measured. When the irradiated cells had completely recovered from potentially lethal damage, they were again exposed to radiation and allowed post-irradiation recovery. The procedure was repeated three times. By use of a quantitative approach – describing the process of recovery as a decrease in the effective radiation dose –, the probability of recovery per unit time and the proportion of irreversibly damaged cells were quantitatively estimated. It was shown that the irreversible fraction of cell injury was increased after repeated exposures to gamma rays, from 0.4 after the first irradiation to 0.7 after the third exposure. The effect was more clearly expressed after exposure to densely ionizing radiation, the corresponding values being 0.5 and 1.0. In contrast, the recovery constant did not depend on the number of repeated irradiations and only slightly depended on radiation quality. It is suggested that the process of recovery from potentially lethal radiation damage itself is not impaired after repeated exposures to both low- and high-LET radiations, and the decrease in the ability of the cell to recover from radiation damage is mainly explained by the increase in the proportion of irreversibly damaged cells.

1. Introduction

The relative biological effectiveness (RBE) of ionizing radiation with a high linear energy transfer (LET) is known to be dependent on the function of genes controlling cell recovery from radiation damage. In particular, for unicellular organisms of various origins the RBE was greater for cells capable of recovery than for cells with a reduced ability to recover, or completely missing such ability [1–6]. In other words, RBE is determined both by the probability of primary damage production (physical events) and the ability of a cell to recover from radiation damage (biological events). There seems to be common agreement that high-LET radiation, compared with low-LET radiation, produce a higher proportion of damage that is considered to be irreversible [7–10]. This is an important case for the recovery from potentially lethal radiation damage observed in irradiated mammalian and yeast cells. More detailed patterns of such recovery at the cellular [11–13] and molecular [14–16] levels are well-known for yeast cells. Furthermore, the ability of eukaryotic cells to recover from radiation damage was first discovered in experiments with yeasts [17]. If irradiated yeast cells are held in liquid non-nutrient medium at 30 °C before plating onto a growth medium, their survival is increased. This phenomenon is now known as liquid-holding recovery (LHR) [18]. The quantitative approach describing the recovery process as a decrease in the effective dose is well-known [11] and it was applied for yeast [19,20] and mammalian cells [21] exposed to ionizing radiation combined with various chemical and physical agents. This approach allows one to determine whether the process of recovery after combined actions is destroyed or damaged itself, or whether the decrease in the rate and extent of recovery is entirely due to an increase in the fraction of irreversible damage.

There are very little data on the analysis of radiosensitivity and the ability of cells to recover after repeated exposures, which are often used in practice. For example, Korogodin [11] analyzed radiosensitivity and LHR after repeated exposures of yeast cells only to low-LET radiation. Similar studies have not been conducted with

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densely ionizing radiation that causes more severe, irreparable damage. The repeated irradiations were shown not to change the radio-sensitivity of the cell, but to result in a reduced ability of the cell to recover, which was expressed both in the extent and the rate of recovery [11]. These effects may arise either from damage to the recovery process itself, or from the formation of irreversible damage that cannot be repaired at all. Both processes may take place at the same time. However, data distinguishing these possibilities are lacking in the literature.

The data related to reduction of the ability of cells to recover from sub-lethal radiation damage after repeated irradiations are well-known for cultured mammalian cells. The progressive reduction in the extrapolation number up to complete disappearance of the shoulder in the sigmoid dose–response curve is also well-known for high-LET radiation [22]. The cellular ability to recover from potentially lethal damage after repeated exposures to low- and high-LET radiation has hardly been studied. Qualitatively, most radiobiological responses of yeast and mammalian cells are similar to each other. For example, survival curves for haploid yeast and mammalian cells are exponential. Conversely, survival curves for diploid yeast and mammalian cells are sigmoid. Therefore, yeast cells are a convenient model for studying the cellular ability to recover from potentially lethal damage after repeated exposures to radiation of different quality.

Our investigation has been undertaken to examine these issues in yeast cells. In this study, the liquid-holding recovery will serve as an indicator of the cellular repair activity. Understanding of the underlying mechanism will hopefully lead to a better insight into the relationship between radiation of various quality and cell recovery from potentially lethal radiation damage. Thus, the main goals of this study were (a) to study radiosensitivity and recovery of yeast cells after repeated exposure to low- and high-LET radiation; (b) to answer the question whether or not high-LET radiation affects the recovery process itself, or whether it only produces a higher level of severe and irreversible damage that cannot be repaired at all; (c) to elucidate the RBE of high-LET radiation after repeated irradiation of yeast cells.

2. Material and methods

The yeast *Saccharomyces cerevisiae* (strain XS800, diploid) in the stationary phase of growth was used for these experiments. To attain this phase, cells were incubated before irradiation on a solid nutrient agar during four days. After attaining the stationary phase of growth, the cells were washed with distilled water and a cell suspension of 5×10^7 cells/ml was prepared. Cells from the same stock solutions were irradiated with ^{60}Co γ-rays (23 Gy/min) and with ^{239}Pu α-particles (20 Gy/min). The LET of the particles reaching the cell monolayer was estimated to be about 120 keV/μm. Just at this LET value the maximum in RBE–LET relationship was observed for most eukaryotic and some prokaryotic unicellular organisms. The small range of α-particles necessitated the use of a monolayer of yeast cells for irradiation: 0.02 ml of the cell suspension was placed on the surface of a non-nutrient agar, and the water from this drop of suspension was evaporated. The viability of the yeast cells after γ-irradiation was identical for cells on the agar surface and for the cells in the water suspension. All irradiations were carried out in air at room temperature (20 ± 2 °C). Immediately after irradiation, part of the sample was plated on nutrient agar plates for the assay of colony-forming ability. Another part of the irradiated cell suspension was placed in conditions suitable for the LHR. After three days (delayed plating) their colony-forming ability was checked again. LHR was carried out in water suspension at 30 °C. After the full LHR of cells irradiated with γ-rays at 1200 Gy and α-particles at 400 Gy, the suspension was again irradiated with the same increasing doses. This procedure was then repeated a third time. At the end of the treatment, the samples were further diluted and plated on nutrient agar to assess colony-forming ability. The survival response was determined by colony counts at the end of 5–7 days of incubation at 30 °C. Each data point represents the average survival on three to six Petri dishes, each containing 50–200 clones. Experimental points in all the figures have errors of 2% or less, that is, approximately the size of the points. Dose–effect curves have been drawn by visually fitting the experimental points. Dosimetry, irradiation and other details have been published previously [3,23,24].

To observe the recovery kinetics, another part of the irradiated cell suspensions and the untreated controls were placed under conditions suitable for the LHR, and their colony-forming ability was determined as a function of the incubation time in recovery conditions (delayed plating). LHR was carried out in a water suspension at 30 °C without constant agitation (the cell concentration was 10^6 cells/ml). The dose–response curves and recovery kinetics were independent of whether the cell suspension was prepared with 0.07 M phosphate buffer or with distilled water. Survival response on immediate and delayed plating was determined on the basis of the colony counts obtained at the end of 5–7 days of incubation at 30 °C. The counts were checked again after a further period to ensure that the final score had been reached.

During the LHR process a number of the primary radiation damages is eliminated, resulting in an increased cell survival. This can be considered as a reduction of the initial dose D_1 to a certain effective dose $D_{\mathrm{eff}}(t)$ which is proportional to the mean number of the residual damages, both reparable and irreversible, after a recovery for t h. It has been demonstrated for yeast cells [19,20] that the decrease in the effective dose $D_{\mathrm{eff}}(t)$ with the recovery time t may be described as follow

$$D_{\mathrm{eff}}(t) = D_1 \left[K + (1-K) \times e^{-\beta \times t}\right], \tag{1}$$

where β is the recovery constant that characterizes the probability of the recovery per unit time. In other words, the recovery constant is approximately equal to the fraction of the reversible radiation damage recovering per unit time. The fraction of radiation damage K is the irreversible component of radiation damage, which can be determined as

$$K = \frac{D_{\mathrm{eff}}(\infty)}{D_1}, \tag{2}$$

where $D_{\mathrm{eff}}(\infty)$ is the effective dose corresponding to the plateau of the recovery curve, which is proportional to the mean number of the irreversible damage. The ratio

$$K(t) = \frac{D_{\mathrm{eff}}(t)}{D_1} \tag{3}$$

reflects the relative part of the primary radiation damage that has not been repaired during t h of recovery. It follows from Eq. (1) that

$$\beta = -\frac{1}{t} \ln \frac{D_{\mathrm{eff}}(t) - D_{\mathrm{eff}}(\infty)}{D_1 - D_{\mathrm{eff}}(\infty)}. \tag{4}$$

Designating

$$A(t) = \frac{D_{\mathrm{eff}}(t) - D_{\mathrm{eff}}(\infty)}{D_1 - D_{\mathrm{eff}}(\infty)}, \tag{5}$$

we have

$$\beta = -\frac{\ln A(t)}{t}. \tag{6}$$

In biological terms, $A(t)$ reflects the proportion of the reparable damage that has not been repaired after t h of recovery. Thus, knowing the survival and recovery curves after cell exposure to low- and high-LET radiation, one can calculate the corresponding values of