Radiobiología

Revista electrónica

ISSN 1579-3087

http://www-rayos.medicina.uma.es/rmf/radiobiologia/revista/radiobiologia.htm

http://www-rayos.medicina.uma.es/rmf/radiobiologia/revista/numeros/RB2(2002)18-22.pdf

Radiobiología 2 (2002) 18 – 22

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Recibido 10 marzo 2002; aceptado 23 mayo 2002

Edita: Grupo de Investigación de Radiobiología. Dpto. Radiología y Medicina Física. Universidad de Málaga (España)

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Abstract

It is known that low doses of ionizing radiation called conditioning doses may induce resistance in exposed organisms to higher doses called challenging doses, and applied after a known period of time. Mechanisms involved in this phenomenon called Adaptive response are diverse and complex. The most important are the activation of DNA-repair enzymes and nuclear recombination processes. An aqueous suspension of the Baker´s yeast Saccharomyces cerevisiae type strain was used as "target". Adaptive response was verified by measuring the population survival in a wide range of challenging doses. Inductors of radio-resistance or conditioning doses were (0.44?0.03) Gy, and the lag time between them and the challenging doses was 2 hours at room temperature.

Key Words: *Saccharomyces cerevisiae, ionizing radiation, adaptive response, low doses effects.*

INTRODUCTION

High doses of ionizing radiation produce detectable clinically damage on living organisms or non-stochastic effects, while those caused by low doses are called stochastic effects because the quantification of their damage is not simple. At the same time, low doses of radiation probably induce mechanisms of resistance through which irradiated cells become radio-resistant to higher doses. These data suggest the possibility of an overestimation of stochastic effects risks to low doses due to the ignoration of adaptive response (AR) processes.

Adaptive response of irradiated cells is a phenomenon produced after the irradiation and depends on the complexity of the organism or cell assayed (Joiner,1994). AR leads to an acceleration or optimization of the cellular repair mechanisms.

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When a relatively high dose is applied these mechanisms are activated and it is observed a lower amount of residual damages than when it is not applied a conditioning or activating dose (IAEA,1997).

After exposure to the conditioning dose, gene activation occurs followed by an accelerate production of enzymes responsible of DNA repair and the delay of the cell cycle (not like a central mechanism but as a consequence of the irradiation). In addition, other mechanisms involved in the radiation damage repair could be activated: detoxification of free radicals and activation of membrane receptors stimulating cell proliferation (IAEA,1997).

The delay of the cell cycle may be called a repair mechanism but strictly, involves itself many of these mechanisms. Spending more time for division, the cells can achieve or improve with high fidelity their DNA repair through constitutive mechanisms others that repair lesions before replication or division.

The variation of radio-sensibility during cell cycle would seem a good argument to explain this phenomenon (Joiner,1994). However, some experiments have demonstrated that this is not the case because cells under arrest show similar adaptive response, therefore the best explanation would be that the first dose induces the activation of repair enzymes (Hower and Cowie, 1978).

Low LET radiation and the free radicals damage are the most effective triggering agents of radioprotection, because they induce single strand breaks in DNA or ionizations, considered the main initiating event of repair mechanisms. Some studies show that little doses of hydrogen peroxide (Sengupta and Bhattacherjee, 1988), and heat (Boreham and Mitchel, 1994), also may induce protection against post-irradiations, supporting the idea that oxidant species and single strand breaks are the major inducers of the response.

The aim of this work was the induction of AR, using different irradiation schemes, over an easy handling unicellular organism and eucaryotic model like the yeast Saccharomyces cerevisiae.

MATERIALS AND METHOD

Strain. Saccharomyces cerevisiae (Baker's yeast) type strain.

Sample Preparation. Inoculums. Pure cultures of Saccharomyces cerevisiae were incubated in test tubes containing YM (Yeast Extract, 3 g/L; Malt Extract, 3 g/L; Peptone, 5 g/L; Dextrose, 10 g/L; Agar, 2 g/L) as the growth medium during 24 h. at 27ºC. Working samples were obtained by spreading 2 or 3 cell loops in 100 ml of sterile distilled water (Mother Suspension, SM) and fractioned on 2 ml samples to be irradiated. Cell concentration on SM was 106 cells/ml in all cases. The percentage of cell buds in these cultures was always kept below 4%.

After irradiation, the samples were maintained in refrigerator (4-10°C) 1 to 4 days until analysis.

Irradiation. The type of ionizing radiation employed in sample irradiation was gamma rays. Electrons beam of 2.5 MeV of an Electron Linear Accelerator (LINAC, CAB) collide against an Aluminium plate (wide 0.6 cm) and produce ?-rays by "Bremsstrahlung" effect. Samples were located 60 cm behind this plate, at room temperature, and were constantly rotated to achieve an homogeneous irradiation. Dose rate was (0.44 ? 0.03) Gy/min (with electron current of 4 µA and 100 pps).

Thermoluminiscence dosimeters of Lithium Fluoride (TLD) were used for measuring radiation dose, because they behave similarly to biological tissue under irradiation.

Cell Surviving and Counting. After each irradiation, the number of viable yeast was registered. The cells were previously stained with methylene blue (2 mg/ml in 0.5 % ethyl alcohol and 99.5 % distilled water), with a final dye dilution of 8.5 µl per ml of sample.

The samples were homogenized in a rotary shaker after dye addition during 1 or 2 minutes (Vortex Decalab). A little drop of stained sample (0.04 ml) was put on wet clean slides to be observed by optical microscope for cell counting. The number of died cells in 500 cells was recorded. To decrease statistical error several measures were performed in each sample.

The surviving fraction was calculated in relation to the amount of viable cells of a control sample (non irradiated) extracted directly of the SM.

Counting of colony forming units (CFU) was first applied and although similar results were obtained, miss data were higher than when applying the staining method. Therefore the latter protocol was preferred.

Data and Figures. Each experiment was made at least twice with independent samples (coming of different cultures). Error bars are shown in all figures except where the error is less than or equal to the symbol size.

RESULTS AND DISCUSSION

Survival to Radiation. Vital staining was performed in irradiated samples without conditioning dose. These results and the previously obtained by the CFU method are shown in Fig. 1 (Durand et al, 1999).

The representative parameters of the radio sensibility of a strain may be LD50 or LD30 (Dutta et al., 1998). In this work we measured the Lethal

Dose 50 (LD50 = 50% of survival) as a sensibility parameter to radiation. LD50 ascertained was (60±1) Gy in the irradiation conditions detailed above, Fig. 1. This value remained constant for all assays.

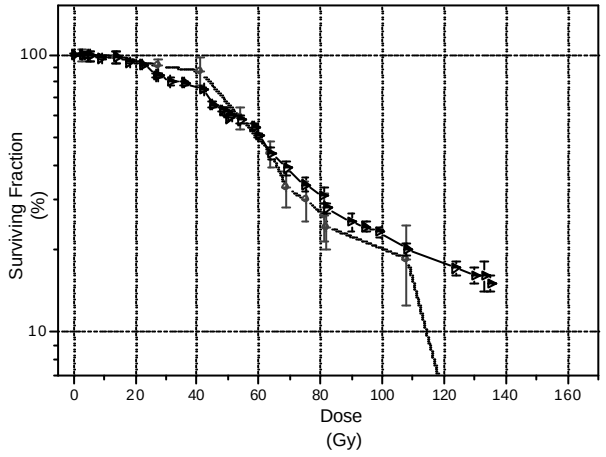

Fig. 1 Surviving Fraction vs. Radiation Doses, quantified by vital staining method (solid triangles) and CFU counting (circles).

Adaptive Response. Designed method to demonstrate AR consisted on short irradiations of very low conditioning doses (about 1 Gy), whose lethality was almost null, Fig. 1. After a certain period of time, denominated waiting time or adaptation time, the samples were irradiated with higher doses of known lethal effect.

To determine if adaptation took place, survivals of pre-conditioned samples and non-conditioned samples were compared.

Initially, irradiations were performed with increasing waiting times of 30, 45 and 60 or 120 minutes, based on Saccharomyces cerevisiae's generation time (g = 90 min). No significant survivals differences appeared, which permitted to observe the adaptive response (Fig. 2).

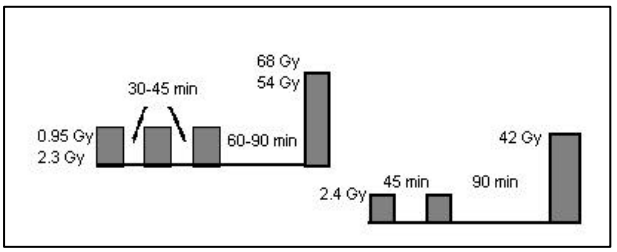

Fig. 2 First Schemes used looking for Adaptive Response.

Scheme described in Fig. 3 was set up, differences between survivals of pre-irradiated, or conditioned, and non-conditioned samples were observed. These first differences were determined with the CFU method.

Samples with a conditioning dose of 4.6 Gy and waiting time of 2 hours showed a survival of 100±5 %, while non-conditioned samples showed 83±4 % survival.

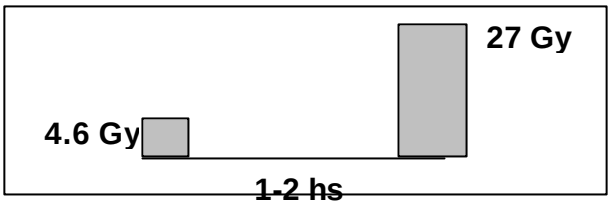

Fig. 3 Irradiation and conditioning scheme showing a possible adaptation to radiation.

Due to these differences it was designed the scheme showed on Fig. 4, wherein conditioning doses were (0.44±0.02) Gy (equivalent to 1 min. of irradiation) and lethal or challenging doses varyied over all the doses range. With this scheme, vital staining method showed remarkable differences between pre-conditioned and non-conditioned samples, setting the previous conditioning dose as a necessary initiator of adaptive response phenomenon.

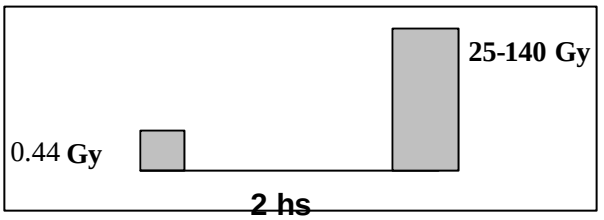

Fig. 4 Final conditioning scheme.

Yeast adaptation to ionizing radiation determined with this preconditioning treatment and comparison between survivals with and without conditioning dose respectively, could be observed in Fig. 5.

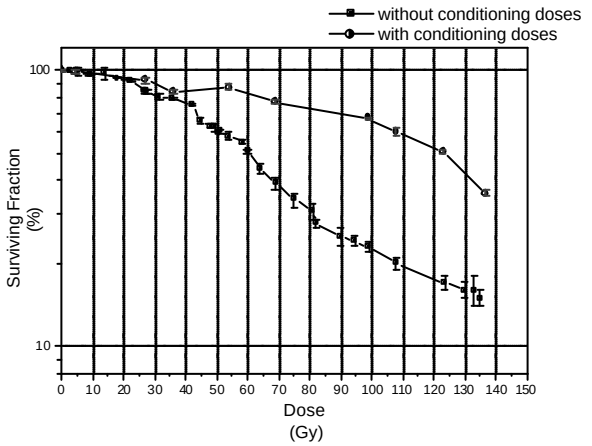

Fig. 5 Comparison between survivals with and without adaptive response.

There is no data available on similar experimental conditions (Tº) but Baker´s yeast

resistance to non-ionizing radiation induced by ionizing radiation conditioning high doses, i.e. under radiation stress, was already observed (Dutta et al, 1998). The authors applied 54 Gy of gamma radiation (10% survival) as conditioning dose and observed increased radio-resistance to a latter dose of UV radiation.

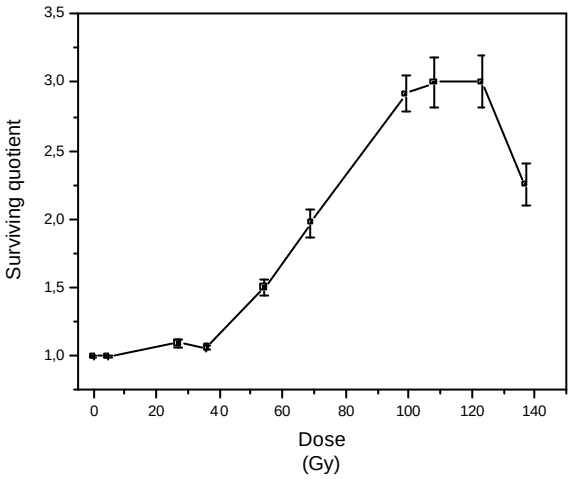

Fig. 6 Quotient between surviving fractions, conditioned and non-conditioned into doses range where Adaptive Response was observed.

In Fig. 6 it could be observed the quotient of surviving fractions shown in Fig. 5. This quotient presents a peak at 110 Gy approximately. The results obtained for the surviving quotient suggest that at high doses, radiation induce more damages than those the process of adaptive response can manage to repair efficiently. With lower doses, the natural repair mechanisms of the cell are enough to repair damages successfully.

CONCLUSIONS

The results presented in this study show that low doses of ionizing radiation induce Adaptive Response to higher doses. This phenomenon has been confirmed in a wide range of challenging doses.

These data would contribute to clarify the debate supported by diverse institutions, regulators of radioprotection and nuclear safety agencies, in respect to low doses effects of ionizing radiation.

Radiation survival of yeast cells suspensions (without conditioning) measured in function of the doses has not being extensively studied.

Many irradiation schemes applied to demonstrate Adaptive Response mechanisms had relative success. The conditioning scheme with which this phenomenon was successfully observed consisted on a conditioning dose of (0.44±0.03) Gy and a waiting time of 2 hours, at room temperature, until the application of the challenging dose.

Under the conditions detailed above, it could be concluded that application of dose around 1% of LD50 increases the radio-resistance of Saccharomyces cerevisiae to posterior doses of ionizing radiation.

The experimental design developed in this work, and its goal of studying cell response to radiation, would permit to continue researching on the ionizing radiation low doses effect on yeasts and other cells from mammals, humans, vegetables and insect origin.

RESUMEN

Este trabajo tiene como objetivo poner de manifiesto el fenómeno de Respuesta Adaptativa, por el cual se induce radioresistencia cuando se exponen células vivas a bajas dosis de radiación ionizante. Estas dosis bajas (condicionantes) pueden inducir resistencia en los organismos expuestos a dosis más altas (de prueba) aplicadas tras un cierto tiempo. Los mecanismos involucrados en este fenómeno de respuesta adaptativa son variados y complejos. Entre ellos se destacan una activación de enzimas de reparación de ADN y procesos de recombinación nuclear.

Una cepa pura "wild type" de Saccharomyces cerevisiae en suspensión acuosa se ha utilizado como muestra "target". Se verificó la respuesta adaptativa midiendo la supervivencia celular en un amplio rango de dosis de prueba. Las dosis condicionantes, inductoras de radio-resistencia, fueron (0.44?0.03)Gy y el tiempo entre las mismas y las de prueba fue de 2 horas a temperatura ambiente.

Palabras clave: *Saccharomyces cerevisiae, radiación ionizante, respuesta adaptativa, efectos a bajas dosis.*

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