INTRODUCTION

In the last few decades a body of in-vitro experimental evidences has shown the presence of a plethora of so-called “non-targeted” phenomena occurring after low-dose ionising radiation (IR) exposures (including hypersensitivity and induced radioresistance, adaptive response, bystander effect and genomic instability), implying that effects of IR arise also in cells that are not themselves directly irradiated in contrast with the “commonly” assumed basic DNA-centric paradigm of radiobiology [1-3].

In spite of the large body of data available in literature, the basic mechanisms of the “non-targeted” effects and their interplay remain still unclear.

The main aim of the present work, carried out in the framework of the INFN project “TANTARA”, is to contribute to improve the understanding of the cellular and molecular mechanisms underlying the variety of the “low-dose non-targeted effects” and their interplay, with particular emphasis on the IR “adaptive response” (AR), in cultured mammalian cells exposed to a single or combined IR fields or beams. Indeed, it has been reported evidence both in-vitro and in-vivo that cells receiving a “first” low-dose of ionizing radiation (called “priming dose”) and then a higher dose (called “challenging dose”) often respond as less sensitive to the latter one than the cells receiving only the higher one, showing the phenomenon called “adaptive response” to IR [3].

A systematic investigation has then been undertaken to measure, after single (“acute”) or multiple-doses (“priming dose” followed by a “challenging dose”) of a single type or combined IR, as a function of radiation qualities and dose, a panel of biological end-points in AG01522 human fibroblasts.

The preliminary results gathered during the year 2014 are reported and briefly discussed.

MATERIALS AND METHODS

AG01522 human primary foreskin fibroblasts (Coriell Institute, USA) were grown in E-MEM medium supplemented with 15% fetal bovine serum, antibiotics, L-glutamine and non-essential aminoacids and incubated at 37°C in a humidified atmosphere of 5% CO2 in air. Under these conditions cells had a doubling time of about 22 h. 48 hours before gamma-ray or proton irradiations cells were plated as a monolayer on the LNL specially designed stainless-steel Petri dishes [4].

Gamma-ray irradiations have been performed at the Nordion “GammaBeam A150” 60Co panoramic source of the INFN-LNL at a dose-rate of 0.1 Gy/min (for the “priming dose”) and 1 Gy/min (for “acute” and “challenging” doses).

Proton irradiations have been performed at the Radiobiology facility at the INFN-LNL 7MV Van de Graaff CN accelerator. Irradiation facility, beam dosimetry and irradiation modalities have been described in detail elsewhere [4]. Cells were irradiated with “broad beams” of 5 MeV (energy at the cell entrance surface) protons, corresponding to LET value of 7.7 keV/µm, at a dose rate of 1 Gy/min.

Five groups of cell samples have been set-up and processed: control (unirradiated cells); 0.1 Gy of one radiation type alone; 0.25 to 4 Gy (“acute” dose of one radiation type alone; 0.1 Gy of gamma-rays (“priming” dose) followed, after an appropriate time lag (7 hours), by graded (“challenging”) doses (0.25 to 4 Gy) of gamma-rays or protons.

After irradiations, appropriate and well established protocols have been used to process and maintain the cells to evaluate:

• Cell survival, by using the cell colony-forming assay
• Chromosomal aberrations, by using mFISH technique
• DNA damage, by using comet assay
• The modulation of interleukin-6 (IL-6) release, by using ELISA technique

RESULTS AND DISCUSSION

Cell Survival

Colony-formation assay has been used to quantify the cell surviving fraction 17 days after irradiation, as a function of dose and radiation quality, in all the five groups of cell samples considered.

The preliminary data analysis, in terms of the parameters of the linear- or linear-quadratic dose-response curves obtained for all the tested conditions, shows

- no evidence of AR in cell survival curve following the combined exposure “gamma-rays + gamma-rays” when compared to the curve following irradiation with single (acute) dose of gamma-rays
- evidence of AR in cell survival curves following the
combined exposure “gamma-rays + protons” when compared to the curve following irradiation with single (acute) dose of protons

Data analysis is in progress.

Chromosomal aberrations

Cytogenetic damage was analysed by the mFISH technique [5] 48 hours after irradiations using at least 100 metaphase spreads for each irradiated sample.

Each chromosome of a metaphase spread was examined based on its unique fluorochrome profile. Structural chromosome aberrations were classified following the mPAINT system [6].

The results expressed as total chromosome exchanges, fragments and breaks show that:

- as expected, a dose dependent increase of cytogenetic damage for both gamma-rays and protons (0.25 and 0.5 Gy), with protons inducing a higher number of aberrations than gamma-rays.

- in case of exposure to the priming dose of gamma-rays a different behavior was observed based on the type of the “challenging dose”, that is gamma-rays or protons, and the aberration type (exchanges, fragments, total breaks).

- in case of analysis of total breaks irrespectively of the type of aberrations (that is fragment 1 break and exchanges 2 or >2 breaks), the priming dose contributes in determining an increase of damage in the combined treatment “gamma-rays+gamma-rays”, whereas for protons a reduced number of breaks, as produced by 0.25 and 0.5 Gy alone, is observed.

DNA damage

The level of primary DNA damage in the irradiated cells as well as in the un-irradiated ones (control cells) has been evaluated using the alkaline comet assay [7].

The preliminary data analysis of a part of the collected data in the various irradiation conditions shows evidence of AR in the cells following the combined exposure “gamma-rays + protons” when compared to the response following irradiation with (only) protons (acute doses). Complete data analysis is in progress.

Cytokine production measurements

These measurements aimed to investigate whether a pre-exposure to low doses of gamma rays may affect the release of IL-6 by irradiated AG01522 cells (with gamma-rays or protons).

To study the kinetics of IL-6 release, culture media from two independent irradiation experiments with “priming + challenging doses” of gamma-rays were collected and filtered at 0, 2, 4, 6 e 12 hours after the cell exposure to the challenging doses and tested with ELISA. The results showed a similar behavior compared with the ones previously measured after “single dose” exposure of gamma-rays [8]. In the case of the lowest challenging dose tested (0.25 Gy), the amount of IL-6 increased for the whole interval of time investigated compared to both control (0 Gy) and “priming dose” alone (0.1 Gy). On the contrary, after challenging doses of 0.5 Gy and 3 Gy the release of IL-6 was lower than that of the control. In particular, after 3 Gy of challenging dose the amount of IL-6 into the medium was up to 35% lower at both 6 hours and 12 hours.

These results did not show any significant changes in the IL-6 release behavior in human fibroblast pre-exposed with 0.1 Gy after gamma rays and then irradiated with 0.25 Gy or 0.5 Gy of gamma rays compared to the ones not pre-exposed.

A single experiment performed with a priming dose (0.1 Gy) of gamma rays and different challenging doses (0.25, 0.5 and 3 Gy) of protons showed kinetic releases of IL-6 with a different temporal behavior and different modulations. Such data need to be confirmed.

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