Biological Effects of LLIR and Normal Oxidative Damage: The Same or Different?

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ABSTRACT

We are establishing a laboratory capable of performing *in vitro* experimentation over a wide range of oxygen partial pressures (from 2.5 to 95% O_2). This 38-fold difference will result in dissolved O_2 concentrations ranging from slightly hypoxic, through physiological (4.3% O_2 at the elevation of Los Alamos), to "normal" atmospheric cell culture conditions (19% O_2), and on to clearly hyperoxic. Taking the physiological O_2 concentration as the appropriate control condition, we will examine and compare the consequences of inducing genetic damage either by level ionizing radiation (LLIR) or temporary exposure to elevated O_2. We will determine if transient oxidative stress induces the same genetic effects, such as DNA base damage, chromosome aberrations, HPRT mutation, and transformation, as exposure to ionizing radiation. Furthermore, we will determine if important cellular responses, such as bystander effects, the adaptive response, and genomic instability, can be induced by LLIR without the additional oxidative stress imposed by atmospheric O_2, or if the threshold dose for initiating these effects changes. The work will determine whether, and to what extent, oxygen concentration affects radiobiological studies, and will shed light on the question of whether the biological effects of LLIR and normal oxidative damage are the same or different.

BACKGROUND

Low LET radiation produces most of its effects through the generation of reactive oxygen species (ROS), *i.e*. the so-called "indirect effect". These ROS are not unique to radiation exposure, but are also produced continuously during the course of aerobic metabolism. *In vitro* radiobiological experiments are conducted almost exclusively under normal atmospheric oxygen, a value that exceeds the *in vivo* physiological oxygen concentration by about six fold at sea level. Thus the true cellular response to low LLIR may be masked or distorted by an unnaturally high level of oxidative lesions imposed by non-physiological O_2. This situation calls into question the soundness of currently accepted radiobiological experimental methods, especially as they relate to science-based risk assessment.

RELEVANCE TO EM NEEDS

- Epidemiology alone is insufficient to estimate risks associated with LLIR with confidence. Yet it is LLIR that is of the most concern in mitigating the environmental effects of the "cold war legacy".

- Incorporation of radiobiological principles offers policy makers a means to improve LLIR risk assessment. These principles must be established under realistic exposure conditions to be defensible.

- Non-physiological oxygen concentration may affect the results of radiation experiments. It is the goal of this project to examine genetic damage, and cellular responses to that damage, induced by LLIR under physiological O_2.

- One important question to be answered: Does LLIR produce biological effects that are the same as, or different from, endogenous oxidative damage?

OXIDATIVE STRESS LABORATORY

Why do we need an oxidative stress laboratory?

- The partial pressure of O_2 may affect the outcome of radiobiological experiments.
- An oxidative stress laboratory allows pO_2 to be treated as an experimental variable.

What will an oxidative stress laboratory do?

- It will have the equipment needed to culture cells in O_2 levels ranging from 2.5% to 95%.
- It will establish procedures for reproducible culture at these O_2 levels.
- It will have the capability to analyze several indicators of intracellular oxidative stress. These include:

  base damage (8-OH-dG; HPLC),

  fluorescent and luminescent ROS indicators.
- It will be sufficiently flexible to accommodate a wide range of experiments. For example:

  multiple biological endpoints,

  low dose rate exposures,

  non-radiation experiments.

RATIONALE

- Does LLIR produce biological effects that are fundamentally different from those caused by endogenous oxidative damage?

- If it does, then there is a firm rationale for radiation protection concepts that seek to limit radiation exposure to the lowest reasonably achievable level.

- Alternatively, genetic damage induced by LLIR may be essentially the same as endogenous oxidative damage. If so, then LLIR would impose a small, and often temporary, increase in the overall burden of genetic damage. This condition would not differ qualitatively from variations in endogenous oxidative damage that occur naturally in response to stimuli such as exercise, dietary change, or infection. In this case radiation protection standards for LLIR might be relaxed.

RESULTS

Model Systems: Two radiobiological phenomena of particular interest to risk assessment are the adaptive response and hormesis. We are establishing model systems for their study.

Adaptive Response: An adaptive response is a protective effect against radiation exposure induced by a low-dose pre-exposure. Our protocol is as follows.

4 cGy → 6 hours → 500 cGy → assay for survival

Results in **Figure 1** show a small but significant adaptive response in CHO-K1 cells exposed to 4 cGy gamma rays.

Hormesis: Radiation hormesis refers to beneficial effects from radiation exposure. The adaptive response may provide a mechanistic explanation. We study this phenomenon with a similar protocol except that the challenge to survival comes from exposure to doxorubicin (Adriamycin), a drug commonly used in chemotherapy. Our protocol is as follows.

4 cGy → 6 hrs → 1.8μg/ml doxorubicin → 2 hrs → survival assay

Results in **Figure 2** show that pre-exposure to 4 cGy causes a 3 to 4.7-fold sensitization to doxorubicin treatment. This unexpected effect is opposite to hormesis.