DEVELOPING NEW RADIOTHERAPY TECHNIQUES USING LINAC BASED GAMMA RADIATION SOURCES

J. D. T. Arruda-Neto[1,2], M. C. Bittencourt-Oliveira[3], A.C.G. Schenberg[4], E. C. Silva[1,3], J. Mesa[5], T.E. Rodrigues[1], F. Garcia[6], M. Louvison[4] and C. R. Paula[4]

[1]Physics Institute – University of São Paulo, São Paulo, SP, Brazil, [2]UNISA – University of Santo Amaro, São Paulo, SP, Brazil, [3] ESALQ – University of São Paulo, Piracicaba, SP, Brazil, [4]Institute for Biomedical Sciences – University of São Paulo, São Paulo, SP, Brazil, [5]São Paulo State University/UNESP, Botucatu, SP, Brazil, [6]Medical Physics Group – Santa Cruz State University, Ilhéus, BA, Brazil

A major challenge in cancer radiotherapy is to deliver a lethal dose of radiation to the target volume while minimizing damage to the surrounding normal tissue. We have proposed a model on how treatment efficacy might be improved by interfering with biological responses to DNA damage using exogenous electric fields as a strategy to drastically reduce radiation doses in cancer therapy. This approach is demonstrated at this Laboratory through case studies with prokaryotes (bacteria) and eukaryotes (yeast) cells, in which cell-killing rates induced by both gamma radiation and exogenous electric fields were measured. It was found that when cells exposed to gamma radiation are immediately submitted to a weak electric field, cell death increases more than an order of magnitude compared to the effect of radiation alone. This finding suggests, although does not prove, that DNA damage sites are reached and recognized by means of long-range electric DNA-protein interaction, and that exogenous electric fields could destructively interfere with this process. As a consequence, DNA repair is avoided leading to massive cell death. Here we are proposing the use this new technique for the design and construction of novel radiotherapy facilities associated with linac generated gamma beams under controlled conditions of dose and beam intensity.

I. INTRODUCTION

In the conventional treatment of cancer, an ideal radiation source would deliver a near-uniform dose to the target and nothing outside it. This paragon is presently unachievable. More realistically, the challenge is to deliver a fatal dose of radiation to the target volume while minimizing damage to surrounding normal tissue. This is partially achieved in therapy with heavy ion beams, but this alternative is still under development as an effective treatment option in most clinical settings[1]. At present, the most common radiotherapy treatments still use γ rays. Several innovative approaches to improve the efficacy of radiation therapy are in progress. A notable example is the search for synthetic compounds that promote the action of p53 proteins[2]. Here we examine the potential of a different treatment strategy designed to interfere with DNA repair processes by means of exogenous physical agents.

Our approach proposes as a very likely possibility that at the DNA damaged sites a static electric field is formed, which works as an instantaneous "marker". Actually, strand breaks do generate static electric fields of quadrupolar nature, as revealed by experiments of perturbed angular correlations of γ rays, an alternative and elegant experimental technique to study the molecular dynamics of DNA[3,4]. This static electric (quadrupole) field persists

until the completion of DNA repair, being responsible for both the orientation of the repairing proteins and their damaged site recognition. Therefore, the "gyrocompass" assuring the correct navigation route of the relevant proteins is the orientation of their dipole momenta along with this static electric field created at the damaged region, as pictorially represented in figure 1a.

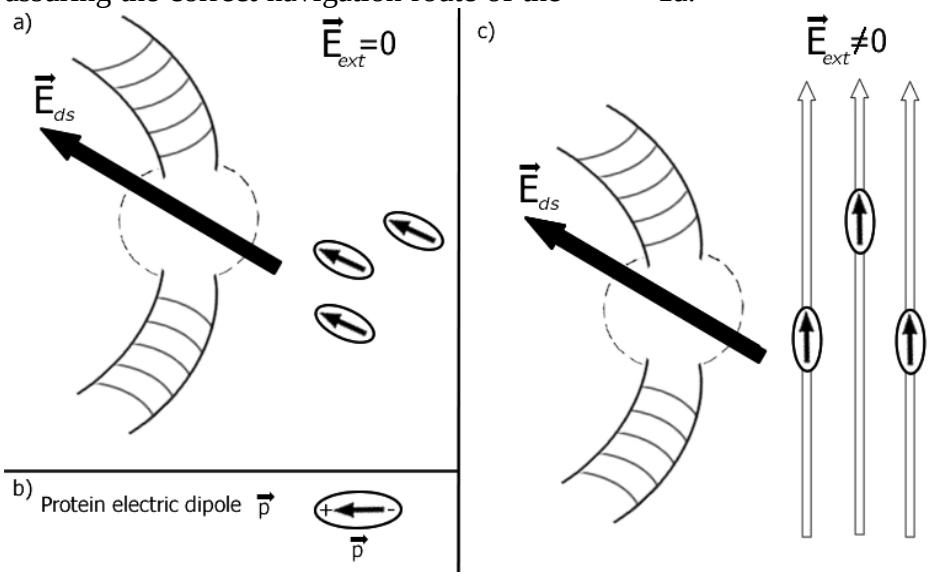


Figure 1 – (a) Pictorial representation of DSB recognition by repair proteins (our hypothesis). These proteins usually have huge electric dipole momenta (represented in *b* with more details), which are oriented toward the damaged site by the static electric field (**E_{ds}**) produced by the electric imbalance at the strand breaks. (*c*) An external static electric field (**E_{ext}**) stronger than **E_{ds}** would reorient the repair proteins displacement all along its direction.

Finally, the model allows one to conjecture that, if a cell population is irradiated with a dose sufficient to induce DNA damage in most of its individuals, and additionally, if an external and static electric field (E-field) with suitable intensity is applied to such a population, the following sequence of events may occur: (1) the repair proteins will tend to gradually align their electric dipoles with the direction of the E-field (see figure 1c); (2) as a consequence, the majority of these proteins will be unable to reach damaged sites in DNA; and (3) lack of repair, particularly of DSB, may lead to cell death. In this sense, cancer radiotherapy combined with the application of E-fields would require much lower doses to eradicate tumors, as addressed below.

Here we present evidence that the exposure of cells to an exogenous electrical field at the time of exposure to ionizing radiation results in enhanced sensitivity to killing by the ionizing radiation. These results have prompted us to the working hypothesis that signaling by DSB is fundamentally electrical in nature, and that perturbing such signaling by the application of an exogenous electric field hinders the sensing of DNA DSB. The experimental strategy employed comprises studies on the yeast *Candida albicans*, as well the prokaryote *Microcystis panniformis*, a cyanobacterium that is remarkably resistant to radiation and heat[5]. Similar considerations led Imamura *et al*. to use the bacterium *Deinococcus radiodurans* in the study of cell-killing induced by the simultaneous action of radiation and heat, in the hope of developing new cancer therapies[6].

II. MATERIALS AND METHODS

We note that all doses referred to in this work were estimated for tissue.

Cell Cultivation and Density Counting

A - *Microcystis panniformis* BCCUSP100 strain (Cyanobacteria) was grown in BG-11 medium[7] for a 14:10 hours (light: dark) photoperiod, at 22 ± 0.5 ºC.

Prior to the beginning of the experiments, the pre-cultures (exponential growth phase) were divided in 10 mL samples and inoculated in 30 mL of new medium in triplicate. Each total volume of 40 mL was housed in a glass tube. The irradiations were carried out with a ^{60}C γ source facility Gammabeam, model 650. All cell samples in glass tubes were simultaneously irradiated with 3 KGy at a rate of 0.94 KGy/h. Immediately after irradiation, the glass tubes were exposed for 2h to a 20 $V.cm^{-1}$ static electric field between the plates of a capacitor. The control tubes were exposed either only to irradiation (3 KGy), or only to the static electric field.

Total cells were enumerated by microscopic counts of culture samples stained with Lugol's 4% solution in a Fuchs Rosenthal haemacytometer. The average number of counted cells ranged from 600 to 1000 in the first and second days after irradiation, respectively. All measurements were performed in triplicate. The results were averaged, and it was found that their dispersion did not exceed 10% (the same was verified in the case of *C. albicans*).

B – *Candida albicans* (strain ICB-12-A) was inoculated in a tube containing Sabouraud agar, incubated at 36 ^{0}C for 20 hours, gamma irradiated with doses from 1 to 4 KGy, and then submitted to a static electric field with net intensity (inside the medium) equal to 180 $V.cm^{-1}$ for 1 hour and 30 minutes. 1 µL of the culture was diluted in a 200µL of PBS, to which 200µL of ethidium bromide (Sigma) and 200µL of fluorescein diacetate (Sigma) were added. A drop of the suspension was set between slide and cover slip and examined under a fluorescence microscope. The number of unviable (red) and viable (apple green fluorescence) cells was counted by means of the fungal-cell viability method in a solution of fluorescein diacetate[8]. One hundred fluorescent cells were counted on each prepared slide, three counts were performed for every sample, and the results are presented as averages of these measurements.

III. RESULTS AND DISCUSSION

Studies with Candida albicans

The survival curves of *C. albicans* cells following exposure to ionizing *radiation with or without an exogenous electrical field* is shown in Figure 2, where the logarithm of the viable cells fraction (S) is plotted as a function of the gamma ray dose (D). The survival curve following irradiation exhibits the well-known initial shoulder, characterized by a slow decrease of viable cells, followed by a faster decrease above ~ 2 KGy. However, when irradiated cells were exposed to the application of a static electric field, the shoulder in the survival curve no longer appeared. The number of viable cells in non-irradiated control samples remained unchanged after the application of the static electric field.

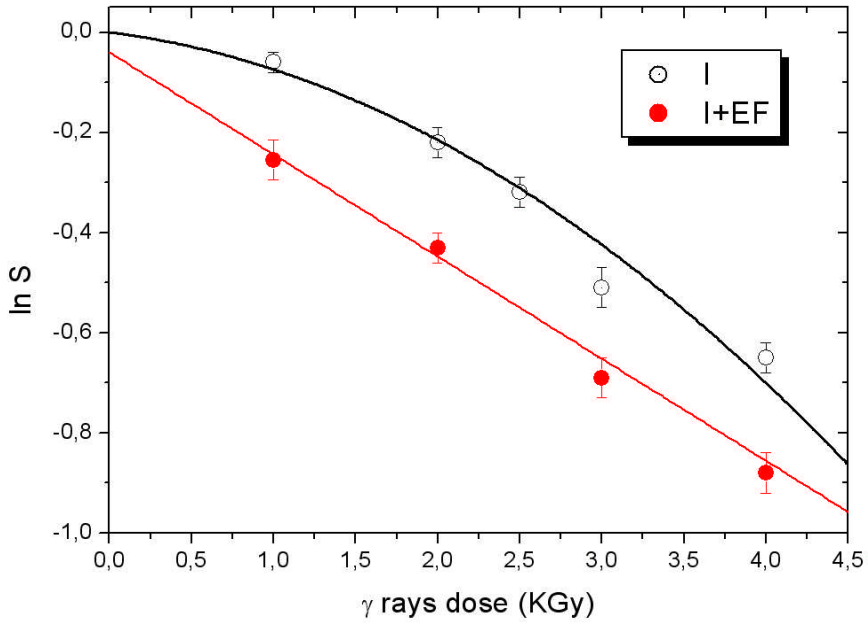


Figure 2 – Survival curves of *C. albicans* as a function of γ-rays doses. Irradiated samples were also submitted to a static electric field of 180 $V.cm^{-1}$ for 1 hour and 30 minutes. Open circles – irradiation only. Full circles – irradiation plus application of an electric field. The fraction of surviving cells is defined as $S = N/N_0$, where N represents the number of cells surviving after treatment, and N_0 is the number of cells before treatment..

The disappearance of the shoulder in the survival curve of *C. albicans* cells exposed to irradiation plus application of an electric field (Figure 2), unambiguously demonstrates that the electric field increased the radiosensitivity of this microorganism. This is an effect similar to that produced by dense ionizing radiation, which produces DSB clusters in DNA molecule that are difficult to repair[9].

Studies with Microcystis panniformis

Because of cell counting peculiarities associated with this experiment it was not possible to observe electric field effects on the survival of *M. panniformis* as a function of dose for a fixed observation time. Instead, we examined the effects of electric field interference on cell growth at a fixed dose of 3KGy. As shown in Figure 2a, the application of an electric field (20 V/cm) did not affect cell growth in control experiments. The average of the relative differences between the two sets of data points is nearly zero. However, when the same electric field was applied for 2 hours immediately after irradiation of the cells, there was a significant decrease in cell growth (Fig. 2b). This is better appreciated in Figure 2c, where the ratio of the two data sets in Figure 2b is plotted. It can be seen that on the second day after irradiation the number of survivors was ~ 12 times greater when no electric field was applied to the irradiated cells.

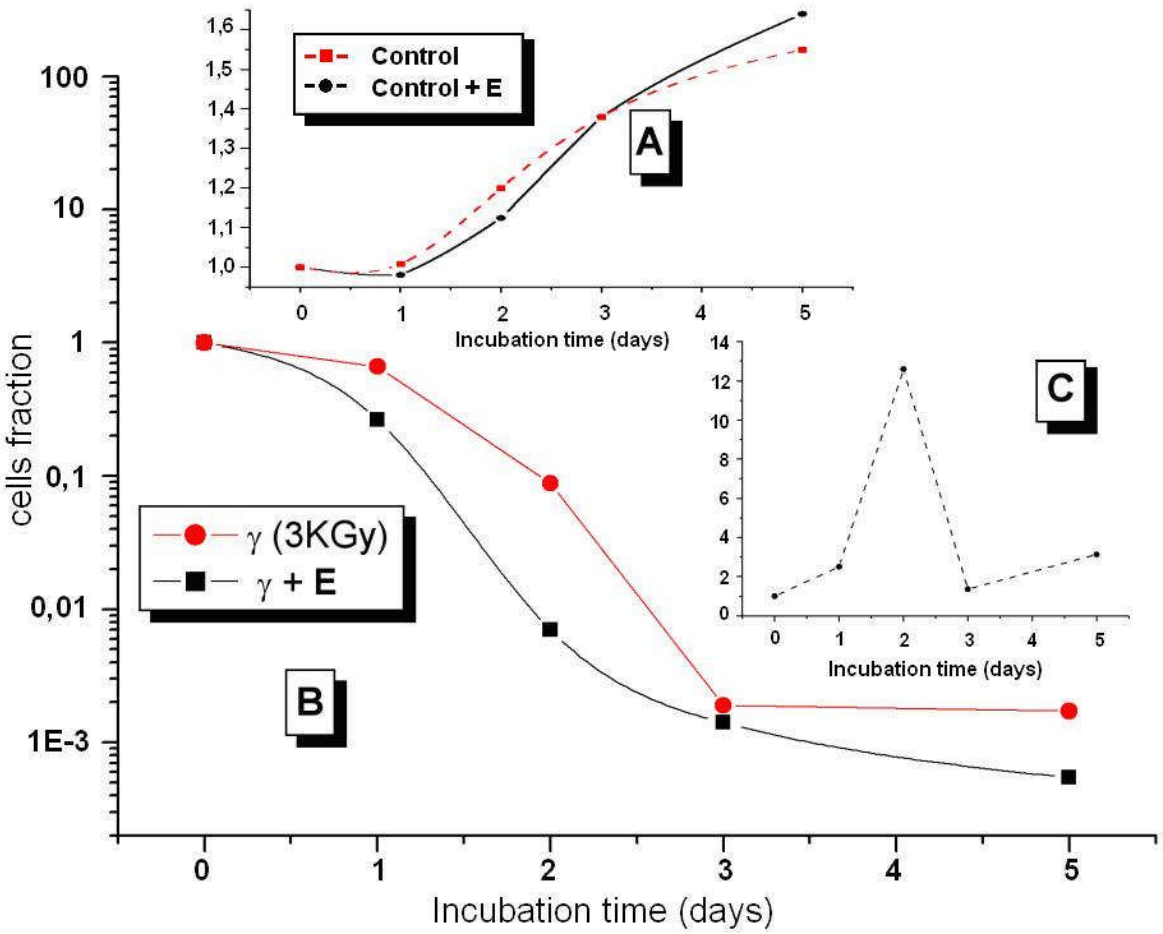


Figure 3 – Growth curves of *M. panniformis*. (a) Growth curves for the control and control plus the application of an electric field, as a function of incubation time. (b) Growth curves following **γ**-rays irradiation with a single dose of 3 KGy, with and without the subsequent application of an electric field. (c) Ratio between the two data sets shown in (b). The ordinate axis of figure 2a (and in figure 2b) provides the fraction of the surviving cells. The 0^{th} day corresponds to the beginning of the experiment. The lines connecting the data points are only to guide the eyes.

IV. CONCLUSIONS

The results from two "case studies" (with a prokaryote and a eukaryote) show that:

1- Application of an electric field following irradiation greatly increases cell death. The effect is similar to the one observed when a source of dense ionizing radiation is used.

2- Electric fields alone have no effect on control samples.

3- Electric fields apparently interfere with repair processes, as evidenced by disappearance of the survival curve shoulder for the eukaryote *C. albicans*.

4- Prokaryotes seem to be more sensitive to the action of electric fields after irradiation.

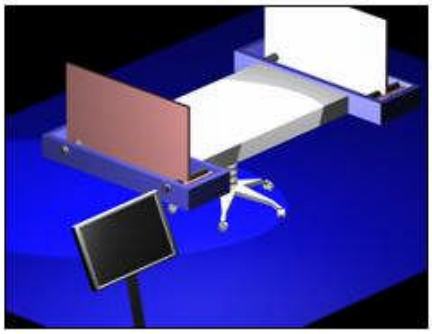
Collectively, these observations suggest, **although do not prove,** that an electric field may prevent the access of repair proteins to damage sites (see figure 1c). Conversely, in the absence of an exogenous electric field, repair proteins find their way to damaged sites by orientation via an endogenous electric field

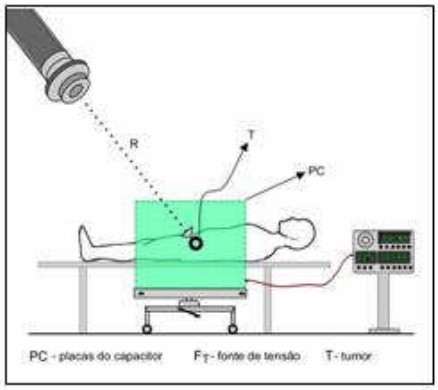
(see Figure 1a) generated during the formation of DSB, and which functions as a navigation cue.

IV.A. Envisaging a new radiotherapy technique

There is active research on biological responsiveness to DNA damage, encompassing and integrating aspects as diverse as the recognition of DNA damage and signaling to activate cellular responses, DNA repair, mutagenesis, programmed cell death, and damage tolerance. It is envisaged that by unraveling complex regulatory pathways leading to the signaling mechanisms that detect DNA damage, parallel technological gains in gene therapy and therapeutic intervention by rational drug design are feasible, offering new strategies for eliminating the unwanted consequences of DNA damage, in particular during the treatment of cancer. In this regard, the combination of radiation with the simultaneous application of weak electric fields in the tumor area may work in this direction. Because of its low intensity, the electric field could be applied externally and directly to the patient, preferentially during and after the tumor irradiation.

In this sense, we propose to test this new technique with human tumor cells, which are available and easy to maintain, by using linac generated gamma beams under controlled conditions of dose and beam intensity. This is easily achievable with gamma radiation obtained from collimated electron Bremsstrahlung in an electron accelerating facility.





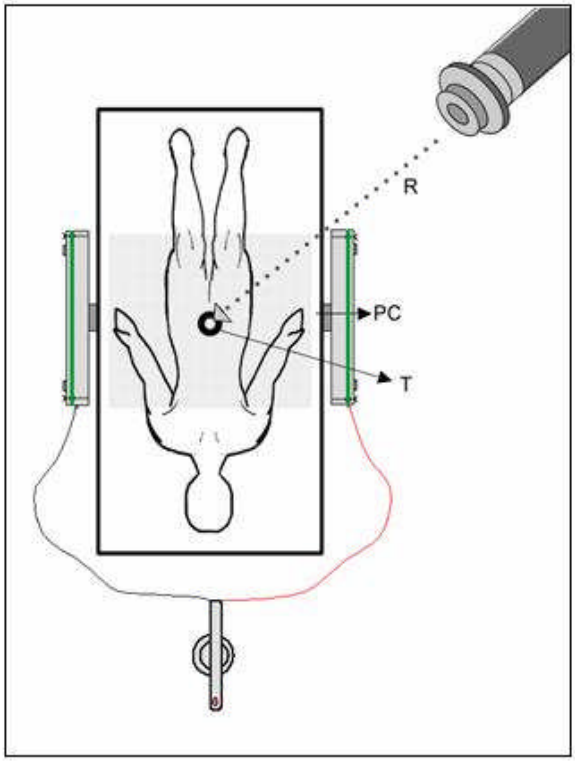


Figure 4 – A proposed new radiotherapy facility, where an external electric field is provided by a capacitor sandwiching the patient.

ACKNOWLEDGMENTS

This work was supported by grants from FAPESP and CNPq, Brazilian agencies for the promotion of science.

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