Using Accelerator Electron Beams To Induce Massive Cell Killing In The Extremophilic Deinococcus Radiodurans

Article ID: WMCPLS00263 ISSN 2051-0799

WebmedCentral building science together

Using Accelerator Electron Beams To Induce Massive Cell Killing In The Extremophilic Deinococcus Radiodurans

Corresponding Author:
Prof. Joao D Arruda-Neto,
University of Sao Paulo, Physics Institute and CEPESq/Uniltalo, Rua do Matao, trav. R, 187, 05508-090 - Brazil

Submitting Author:
Prof. Joao D Arruda-Neto,
University of Sao Paulo, Physics Institute and CEPESq/Uniĺtalo, Rua do Matao, trav. R, 187, 05508-090 - Brazil

Submitted on:22-Oct-2013, 06:28:10 PM GMT

Accepted on:25-Oct-2013, 05:53:07 AM GMT

Article ID: WMCPLS00263

Article Type: Research articles

Article URL: http://webmedcentralplus.com/article_view/263

Subject Categories:BIOPHYSICS

Keywords: Deinococcus radiodurans; electron beams; gamma radiation; Atomic Force Microscopy; DNA fragment sizes; clonogenic death; repairing shoulder depletion

How to cite the article:

Arruda-Neto JD, Segreto HC, Gomez JC, Silva LF, Mendonca TT, Jorge SA, Nieto L, Garcia F, Righi H, Rodrigues TE, Prado GR, Genofre GC.Using Accelerator Electron Beams To Induce Massive Cell Killing In The Extremophilic Deinococcus Radiodurans. WebmedCentral *plus* BIOPHYSICS 1970;-39(1):WMCPLS00263

Source(s) of Funding:

Using Accelerator Electron Beams To Induce Massive Cell Killing In The Extremophilic Deinococcus Radiodurans

Author(s): Arruda-Neto JD, Segreto HC, Gomez JC, Silva LF, Mendonca TT, Jorge SA, Nieto L, Garcia F, Righi H, Rodrigues TE, Prado GR, Genofre GC

Abstract

Background: The extraordinary ability of *Deinococcus radiodurans* to withstand lethal radiation effects has been verified with gammas but not with electron. Results show that electrons are able to severely damage plasmid DNA, shattering it into myriads of fragments, thus motivating the performing of *D. radiodurans* irradiation with electrons to test the limits of its radioresistance *vis-à-vis* new concepts on proteome radiation protection for this bacterium.

Methods: Cells of *D. radiodurans* in stationary growth phase were irradiated with electron of 1.174 MeV and gammas from a ^{60}Co facility. Evaluation of viable colony-forming units of cells was performed by optical cell density measurements at 600 nm, performed in triplicate up to 12 kGy. Samples of *Escherichia coli* were irradiated under the same conditions (positive control). Genomic DNA of *D. radiodurans* irradiated with electrons was extracted, and images of DNA fragments were obtained by Atomic Force Microscopy (AFM).

Results:

1. The 8kGy wide *shoulder* of *D. radiodurans* survival curve, observed in irradiations with gammas, was eliminated by electrons.
2. At electron doses over 2 kGy the number of colonies dropped below the detection limit.
3. Comparison between survival curves shows that *D. radiodurans* and *E. coli* exhibit equal yet low radiosensitivity when exposed to electron beams, and that both also are nearly 100% non-viable at 1.5–2 kGy.
4. *E. coli* is equally highly radiosensitive to gammas and electrons genome is shattered by electrons into large pieces.

Conclusions:

1. Suppression of repairing shoulder demonstrates that electrons are highly cytotoxic to *D. radiodurans*.
2. Intense fluxes of secondary electrons produced by electron beams hamper *D. radiodurans* recovery.
3. *D. radiodurans* is as radiosensitive to electrons as *E. coli*, compelling evidence that protection mechanisms for proteins and genome no longer prevail under this radiogenic stress.

Keywords: *Deinococcus radiodurans*; electron beams; gamma radiation; Atomic Force Microscopy; DNA fragment sizes; clonogenic death; repairing shoulder depletion

Introduction

Deinococcus radiodurans has puzzled biologists since its discovery as a contaminant in corned beef treated with sterilizing radiation [1]. For over a half century its extreme radiation resistance has been associated with its equally high capacity to repair massive DNA damage. Actually, *D. radiodurans* exhibits an extraordinary ability to withstand lethal and mutagenic effects of DNA damaging agents, particularly those from exposure to ionizing radiation producing over a thousand DSB (double-strand breaks) in each cell [2], [3].

Dose-response relationships for clonogenic survival are generally obtained from cell survival data, usually represented by shouldered (sigmoid) survival curves. The conceptual significance of the shoulder in mammalian cells goes back to 1959, as revealed by the seminal work of Elkind and Sutton [4]. According to them, the shoulder is indicative of repair of damage to that electron beams produce intense fluxes of secondary electrons, a severe radiogenic stress.

7. AFM imaging of *D. radiodurans* shows that its

induced DSB, this shoulder is sometimes referred to as *repairing shoulder*. In this sense, the 8 kGy-wide *repairing shoulder* of *D. radiodurans* would point to the

repair mechanism of this bacterium being highly proficient, thus explaining its extremely high radioresistance.

However, the conceptual scenario in radiation biology has drastically been changing. In this regard, a new paradigm for all species was proposed by Krisko and Radman, as lucidly outlined in a recent review [5]. According to them, the proteome rather than the genome is the prime target in radiation-induced cell death. The reason being the fact that cell survival itself depends primarily on vital functions performed by the proteome, while genome integrity is necessary for the perpetuation of the surviving cells. These concepts have been copiously justified elsewhere [Ref. 5 and references therein].

Regarding the bacterium *Deinococcus radiodurans*, more specifically, one is led to conclude that its very high resistance to radiation, and to other sources of oxidative damage, is a consequence of efficient protection against proteome damage. In fact, there has to be in *D. radiodurans* a regulatory interplay between different processes associated with oxidative stress response pathways [3], [6], [7]. These functions, therefore, would manifest themselves as protein protection, preserving the high efficiency of DNA repair enzymes [3], [8].

Thus, survival curves must be interpreted *cum grano salis*, an attempt pursued in the present work. Typical *D. radiodurans* survival curves for acute irradiation with ions of different LET (linear energy transfer), as distinct as He, O and Ar, are shown in Figure 2 of Ref. 9. The curves are characterized by an initial shoulder followed by a relatively abrupt transition to an exponential terminal slope at higher doses (modulated by radiation type). This basic shape is the same as for the low-LET gamma-radiation. The shoulder is nearly insensitive to the radiation-LET, an indication of very efficient DNA damage repair in *D. radiodurans*. This is consistent with current knowledge on both protein protection [Ref. 5 and references therein] and DNA repair in this organism. The latter is homology based, utilizing multiple genome copies, and is in principle error free, resulting in essentially 100% cell survival and absence of mutagenesis up to certain doses [9], [10], [11].

Because of these intriguing characteristics, *D. radiodurans* is high on the agenda of many radiobiological applications and as pointed out elsewhere, the use of this microorganism as a bacterial model for oncology [12] and long-lived non-dividing neurons processes [10] is an appealing possibility. Moreover, exploring mechanisms of *Deinococcal* robustness may well also inspire approaches in anti-ageing research and regenerative medicine [6].

The extraordinary radioresistance of *D. radiodurans* has been verified mostly with the low-LET gamma radiation from ^{60}Co-facilities and Ultra-Violet-C radiation, but not with electron beams from linear electron accelerators (LINAC), another low-LET radiation also widely used in radiotherapy. The reason could be the fact that both radiations have comparable LET.

However, an experiment on fragmentation profiles of plasmid DNA irradiated with gammas and electrons, recently carried out at this Laboratory using Atomic Force Microscopy to determine fragment lengths, showed that the DNA strands were highly shattered when irradiated with electrons [13]. This unexpected finding served as motivation to examine the limits of *D. radiodurans* radioresistance by carrying out viability measurements after irradiation with electrons.

As shown in this work, electron beams from a LINAC facility produce intense fluxes of secondary electrons, constituting thus a severe radiogenic stress to both proteome and genome of D. radiodurans – also addressed in this work.

Finally, a possible extraterrestrial origin of *D. radiodurans* has been conjectured elsewhere [14]. In fact, *D. radiodurans* is one of the most investigated organisms in the experimental test of the *panspermia* hypothesis. According to this hypothesis, bacterial cells are able to be transferred across large distances of interplanetary space and thus "seed" planetary bodies. Any viable life-form putatively traveling from one inhabited planet to another would, therefore, have to cope with cosmic radiation of the interplanetary space, which is constituted mostly by protons and electrons (≈ 92%, in roughly equal numbers). The electron distribution functions in the solar wind approximate Maxwellian energy distribution with average energies in the order of 10 eV [15]. Although these are low energy electrons, the amount of radiation imparted to *D. radiodurans*, while hypothetically traveling from one planet to another, would be very high (order of several kGy) given the long lasting time of exposure.

Materials And Methods

Cultures of *Deinococcus radiodurans*, GY 9613 (R1) wild-type strain, were obtained from a stock kept at the Institute for Radioprotection and Dosimetry/(IRD/RJ), Rio de Janeiro, Brazil. The cells in freezer stocks (glycerol 10%, -80°C) were streaked in solid TGY