# Modeling Space Radiation With Radiomimetic Agent Bleomycin


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## Introduction

Space radiation consists of proton and helium from solar particle events (SPE) and high-energy heavy ions from galactic cosmic ray (GCR). This mixture of radiation with particles at different energy levels has different effects on biological systems. Currently, majority studies of radiation effects on human were based on single-source radiation due to the limitation of available method to model effects of space radiation on living organisms. While NASA Space Radiation Laboratory is working on advanced switches to make it possible to have a mixed field radiation with particles of different energies, the radiation source will be limited. Development of an easily available experimental model for studying effects of mixed field radiation could greatly speed up our progress in our understanding the molecular mechanisms of damage and responses from exposure to space radiation, and facilitate the discovery of protection and countermeasures against space radiation, which is critical for the mission to Mars.

Bleomycin, a radiomimetic agent, has been widely used to study radiation induced DNA damage and cellular responses. Previously, bleomycin was often compared to low low Linear Energy Transfer (LET) gamma radiation without defined characteristics. Our recent work demonstrated that bleomycin could induce complex clustered DNA damage in human fibroblasts that is similar to DNA damage induced by high LET radiation. These type of DNA damage is difficult to repair and can be visulaized by γ-H2Ax staining weeks after the initial insult. The survival ratio between early and late plating of human fibroblasts after bleomycin treatment is between low LET and high LET radiation. Our results suggest that bleomycin induces DNA damage and other cellular stresses resembling those resulted from mixed field radiation with both low and high LET particles. We hypothesize that bleomycin could be used to mimic space radiation in biological systems. Potential advantages and limitations of using bleomycin to treat biological specimen as an easily available model to study effects of space radiation on biological systems and to develop countermeasures for space radiation associated risks will be discussed.

## Evidences

### Bleomycin can induce complex clustered DNA damage like high linear energy transfer (LET) radiation

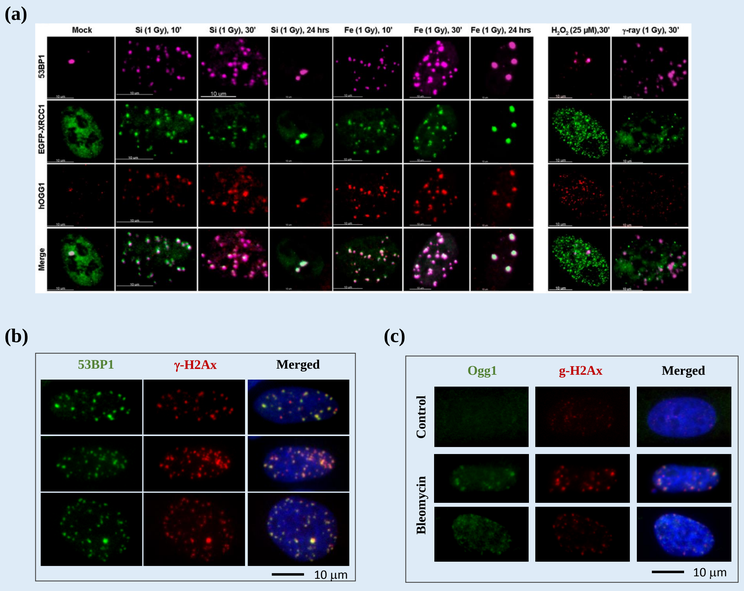
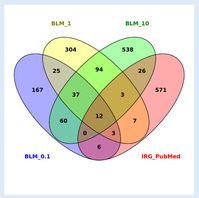



**Figure 1**. (a) High LET radiation induces clustered DNA damage evidenced by colocalization of different DNA damage response proteins, published by A Asaithamby and DJ Chen [1]. (b) Bleomycin induced DNA damage is double-strand break. All cells in (b) were treated with 1.0 mg/ml for 3 hours.(3) Bleomycin can induce clustered DNA damage like high-LET radiation. [2]

### Gene expression profile in confluent normal human fibroblast AG01522 cells after bleomycin treatment have common molecular signatures with ionizing radiation and UV exposure treated cells

**(a)**




**(b)**

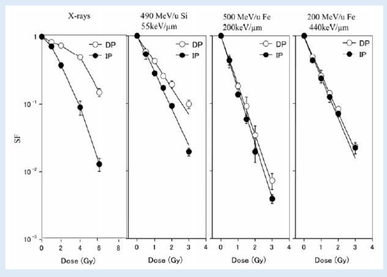
| BLM IPA | IR Pubmed |
|---|---|
| CDKN1A | CDKN1A |
| PCNA | PCNA |
| MDM2 | MDM2 |
| FAS | FAS |
| NFKBIA | NFKBIA |
| EGR1 | EGR1 |
| IL6 | DDB2 |
| | PPM1D |
| | PMAIP1 |
| | POLH |
| | SESN1 |
| | ZMAT3 |

**(c)**

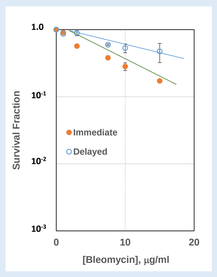

**Figure 2.** (a) Venn diagram of significantly regulated genes in confluent normal fibroblast AG01522 after treatment with bleomycin at (0, 0.1, 1.0 and 10 μg/ml) for three hours were compared with genes associated with ionizing radiation in PubMed database; (b) Genes that are common between IPA bleomycin regulated genes and ionizing radiation responsive genes in PubMed databases. (c) GSEA analysis showed that bleomycin-responsive genes are most enriched with gene sets that are associated with ionizing radiation, where absent data points where in yellow color. Here, "BLM" stands for bleomycin and "IRG" for ionizing radiation responsive genes. [2]

### Cell survival behavior of confluent normal human fibroblast AG1522 after bleomycin treatment is similar to the mixed field radiation exposure.

**(a)**




**(b)**




**(c)**

| | LET (KeV/μm) | R (IP/DP) |
|---|---|---|
| X-ray | 0.3 | 2.8 |
| Si | 55 | 1.4 |
| Fe1 | 200 | 1.1 |
| Fe2 | 400 | 1.1 |
| | | |
| GCR | *17.6#* | *1.75** |
| Bleomycin | *6.64** | 1.99 |

**(d)**

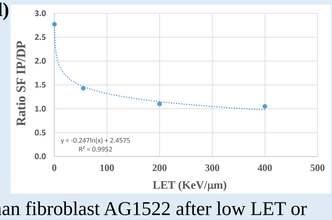



**Figure 3.** (a) Survival curves of confluent human fibroblast AG1522 after low LET or high LET irradiation, published by Cucinotta et al. [3] (b) Survival curves of confluent human fibroblast AG1522 after bleomycin treatment. (c) Ratio of the slopes of survival fraction of immediate and delayed plating. (d) LET energy vs Ratio of survival fraction (IP/DP). Note: clonogenic assays were done after radiation or bleomycin treatment immediately (IP) or delayed for 12 hours (DP). "#" Estimation of galactic cosmic ray (GCR) LET was personal communication from Dr. Ianik Plante. [4, 5] "*" are estimated values.

## Discussion

While reproducing space radiation is not easy with accelerators due to the nature of mixed particles, our recent findings suggest that bleomycin might induce DNA damage that is similar to the exposure to mixed field radiation like space radiation.

First, our recent novel finding suggest that some of the DNA damage induced by bleomycin are clustered DNA damage, evidenced by the colocalization of proteins in different DNA damage response pathways at the DNA damage sites.

Secondly, our study show that cellular response to bleomycin treatment at gene expression level have similar molecular signatures with ionizing irradiation. Several key genes were activated in cells treated with bleomycin and ionizing radiation.

Thirdly, confluent fibroblasts show similar survival behavior that is in between of those of cells exposed to low LET radiation and high LET radiation.

Fourthly, calculation from experimental data and theoretical modeling suggestion that space radiation by galactic cosmic ray (GCR) and bleomycin have similar "effective" LET.

While the need in technology advancement to make it possible to reproduce GCR radiation with accelerators to study its biological effects, I suggest bleomycin could be used to mimic the effect of space radiation on biological systems.

Bleomycin has the advantages in availability, cost, and in certain experiments that may not be done easily with accelerators.

## References:


1. A Asaithamby, B Hu, DJ Chen (2011) Unrepaired clustered DNA lesions induce chromosome breakage in human cells. *Proc Natl Acad Sci.*, **108**, 8293–8298.
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