Comparative Analysis of Ionization Radiation Diagnostics: Micronuclei Versus Dicentric Chromosome Techniques

Brian McNeel & Jude Platukis

Appalachian STEM Academy at Oak Ridge 2022

Appalachian STEM Academy at Oak Ridge

OAK RIDGE National Laboratory

Introduction

Humans are exposed to ionizing radiation (IR) by various sources such as diagnostic X-rays, air travel, natural radiation, and radiological/nuclear terrorism. Exposure to ionizing radiation (IR) can cause cellular damage (DNA double strand breaks) at the chromosome level that could lead to cancer. Determining the extent of IR exposure is therefore critical for assessing the amount of cellular damage. Human T- lymphocytes are ideal for assessing IR exposure because they are highly radiosensitive. Radiation can cause double strand breaks in DNA as it passes through the nucleus and some of these breaks are misrepaired leading to the formation of chromosome aberrations. A previous study by Pajic et al. (2015) analyzed both dicentrics and micronuclei for assessment of absorbed radiation dose in human lymphocytes.

In the absence of physical dosimeters for radiation dose detection, biological dosimeters can be useful for estimating the absorbed radiation dose in accidentally or occupationally exposed humans (Zeegers et al., 2017). Dicentric chromosome displays two centromeres due to misrejoining of two broken chromosomes after IR exposure. Micronucleus formation occurs when a broken chromosome fragment or whole chromosome is excluded from the main nucleus during cell division. Comparing these two biodosimetry techniques for radiation sensitivity and specificity is the goal of this project to improve the accuracy of radiation dose prediction.

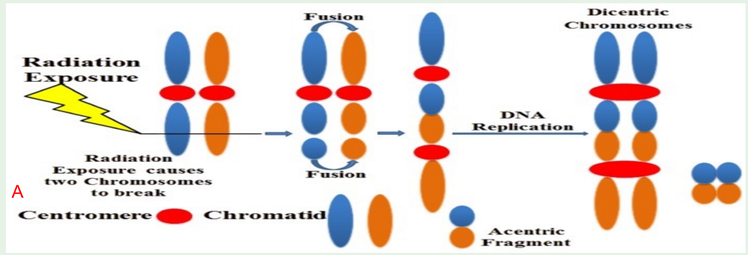


Figure 1: The mechanistic bases for the formation of dicentric chromosomes and micronuclei are shown in Figure 1A & 1B. Figure 1C is a representative metaphase showing a dicentric chromosome (box) and an acentric fragment (circle). Figure 1D shows a micronuclei in a binucleated cell.

Materials and Methods

In vitro lymphocyte culture & Irradiation

Collect 10mL of Blood Sample

Irradiation with X-rays (0-5 Gy; dose rate 2 Gy/min) in complete growth medium

Incubate cells for 48hrs 37 °C in a 5% CO_2

Dicentric Chromosome Assay (DCA)	Cytokinesis Blocked Micronucleus Assay (CBMN)
Add Colcemid (0.1 µg/ml) the last 24hrs of incubation	Add Cytochalasin B (6 µg/ml) the last 28hrs of incubation
Harvest cells 48hrs after the initiation of cultures	Harvest cells 72hrs after the initiation of cultures
Treat with hypotonic solution (0.56% KCl)	Treat with hypotonic solution (0.56% KCl)
Fixation (3x) acetic acid: methanol (1:3) mixture	Fixation (3x) acetic acid: methanol (1:4) mixture
Drop 25-30 µl of cell suspension onto slide & treat RNase A	Drop 25-30 µl of cell suspension onto slide
Stain with Giemsa	Stain with DAPI

Imaging: Perform imaging and analysis of Dicentric Chromosomes & Cytokinesis Blocked Micronuclei

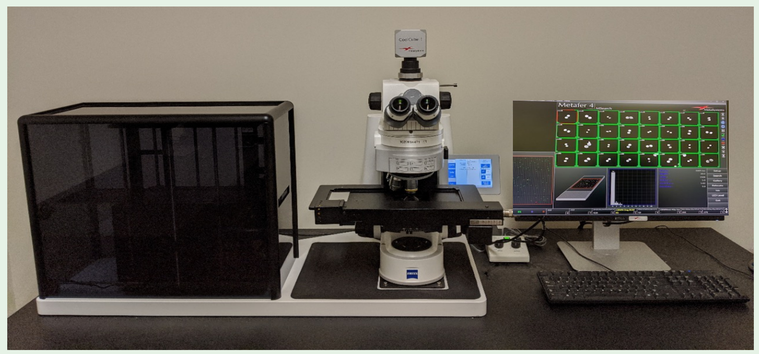
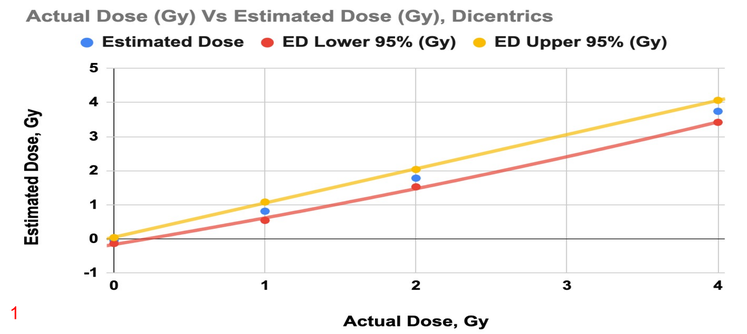


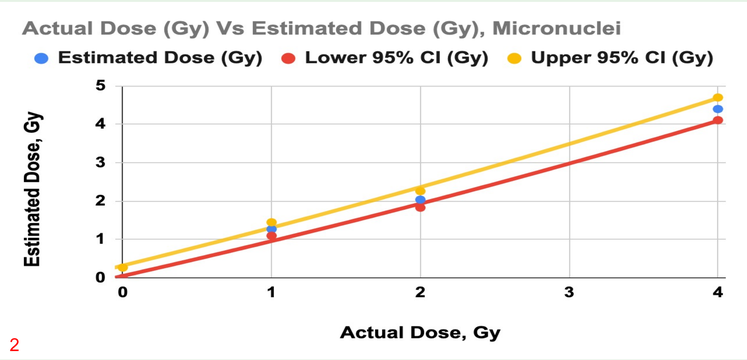
Figure 2: Microscope equipped with ISIS software (MetaSystems Inc. USA) was used for image capture and analysis.

References

- Pajic, J., Rakic, B., Rovcanin, B., Jovicic, D., Novakovic, I., Milovanovic, A., & Pajic, V. (2015). Inter-individual variability in the response of human peripheral blood lymphocytes to ionizing radiation: comparison of the dicentric and micronucleus assays. Radiation and Environmental Biophysics, 54(3), 317–325. https://doi.org/10.1007/s00411-015-0596-3
- Zeegers, D., Venkatesan, S., Koh, S. W., Low, G. K. M., Srivastava, P., Sundaram, N., Sethu, S., Banerjee, B., Jayapal, M., Belyakov, O., Baskar, R., Balajee, A. S., & Hande, M. P. (2017). Biomarkers of Ionizing Radiation Exposure: A Multiparametric Approach. Genome Integrity, 8. https://doi.org/10.4103/2041-9414.198911

Results





Graph 1 & 2: These two graphs model a comparison of predicted biodose (Gy) with the delivered physical dose (Gy). Graph 1 is based on the number of dicentrics per cell and graph 2 is based on the frequency of micronuclei per binucleated cell. In both, a 95% confidence interval was applied. The dose was estimated using standard calibration curves (CBMN: $Y = 0.0013 +/- 0.0035 + 0.018+/-0.0.01*D + 0.02+/- 0.003*D^2$) (Dicentrics: $Y= 0.0019 + 0.0454*D + 0.0621*D^2$) , where Y is the yield and D is the radiation dose.

Conclusions

I. The two biodosimetry methods- Dicentric Chromosome Assay (DCA) and Cytokinesis Blocked Micronucleus (CBMN) assay- used in this study yielded grossly similar estimates of absorbed radiation dose.
II. Biodoses estimated by both DCA and CBMN differed from the actual delivered physical dose by less than 0.5 Gy.
III. Absorbed radiation doses estimated by CBMN were slightly higher than that predicted by DCA.
IV. Both biodosimetry assays can be interchangeably used for absorbed dose estimation in radiation exposed humans.

Acknowledgements

Thank you to Dr. Adayabalam S. Balajee, Maria Escalona, & Terri Ryan. We appreciate the opportunity provided to us by Oak Ridge National Laboratory, Oak Ridge Associated Universities and the Appalachian Regional Commission.