EFFECTS OF LOW-DOSE GAMMA AND NEUTRON RADIATION ON GENOTOXICITY AND CYTOTOXICITY OF RETICULOCYTES IN A MOUSE MODEL

Nghi Phan, Nicole M. McFarlane, Jennifer Lemon, Doug R. Boreham

McMaster University, Medical Physics and Applied Radiation Sciences Unit,
1280 Main St. West, Hamilton, Ontario, Canada. L8S 4K1.

Abstract

Using a successful new automation of micronucleated reticulocyte (MN-RET) scoring, the effects of low-dose (< 1.0 Gy) gamma and neutron radiation on genotoxicity and cytotoxicity of reticulocytes (RET) in a mouse model were investigated. Gamma and neutron irradiation induced significant ($p<0.001$) increases in the levels of %MN-RET and decreases in the levels of %RET ($p<0.001$) as the dose level increased. Increasing dose levels showed that gamma radiation induced significantly ($p<0.05$) more %MN-RET and more %RET than neutron radiation. The results suggest that neutron irradiation may be more cytotoxic (less %RET) than gamma irradiation; however, gamma irradiation may be producing cells with more chromosomal aberrations (more %MN-RET) than neutron irradiation.

1. Introduction

In the event of an accidental or terrorist-related radiological disaster, it is crucial to be able to assess the level of dose exposure and the extent of biological damage. This information is critical in determining the appropriate immediate medical attention, as well as assessing the long-term carcinogenic risk. Traditional biodosimetry techniques such as dicentrics and micronucleated (MN) lymphocytes represent the current gold standards for estimating radiation effects on cells in the peripheral circulation [1]. These assays are performed through microscopic inspection of blood or bone marrow samples, thus are limited by the relatively small number of cells analyzed and by their subjective nature. It is therefore inadequate to triage large populations following a radiological disaster using these cytogenetic damage endpoint assays.

In recent years, a successful automated approach for enumerating micronucleated erthroctye populations has been developed that can potentially overcome the drawbacks of the microscopic approaches. The flow cytometry (FCM)-based system of scoring micronucleated reticulocytes (MN-RET) has been an attractive candidate for radiation biodosimetry purposes [2]. Using the FCM-based MN-RET assay, the present study investigates the effect of low (gamma) and high (neutron) Linear Energy Transfer (LET) radiation on cytogenetic damage to hematopoietic cells in a mouse model. The project aims to confirm the sensitivity of the MN-RET assay, as well as determine various dose-response properties associated with low doses (<1 Gy) of gamma and neutron radiation using the MN-RET cytogenetic damage endpoint.

Previous research has shown that neutrons have high relative biological effectiveness (RBE) values for various biological endpoints, including chromosome aberrations in human lymphocytes [3]. However, there is not much research concerning neutrons effect on young reticulocyte populations

in a mouse model. With a higher RBE than gamma radiation, it is postulated that using the FCM-based MN-RET assay, neutron irradiation will induce greater genotoxic and cytotoxic effects than gamma radiation in mouse reticulocytes.

2. Materials and Methods

2.1 Reagents

The reagents for the FCM-based MN-RET assay were supplied by Litron Laboratories Rochester, NY, via their MicroFlow® Mouse Micronucleus Analysis Kit. The kit contained anticoagulant solution, fixative solution, methanol-fixed biological standards, washing/dilution buffer, anti-CD71-FITC antibody, nucleic acid staining solution, RNase solution, instruction manual, and CD-ROM with FCM data acquisition and analysis template files for use with a Beckman Coulter EPICS XL flow cytometer (Beckman Coulter, Fullerton, CA).

2.2 Animals

Fifty female C57Bl/6N mice aged 7-8 weeks were obtained from the Central Animal Facility at McMaster University (Hamilton, Ontario). Animals were allowed to acclimatize for at least three weeks prior to irradiation. Animals were housed in groups of five in solid-bottom polycarbonate cages with stainless steel wire-bar lids. Bedding consisted of wood chips. Food and water were available *ad libitum* throughout acclimation and post-irradiation periods. Mice were monitored daily. The food, water, and bedding were restocked/changed every other day. The experiment had ethical approval and animals were treated in strict accordance with animal welfare guidelines. Five days prior to the time of irradiation, the mice were conditioned to the environment they would experience during the irradiation process. Mice were 11-12 weeks old at the time of irradiation.

2.3 Irradiation Conditions

Mice were randomly assigned to 10 groups of 5 mice. Five groups were treated with total body irradiation with McMaster's Taylor Source, a 662 KeV Cs137 gamma, low LET source. The other five groups were treated with total body irradiation at McMaster's Tandem Accelerator, a 2.5 MeV, 500 μA neutron, high LET source. Mice were irradiated two at a time except for the fifth mouse in the group. While irradiated with gamma radiation, mice were contained in a 50 mL plastic conical tube. During the neutron irradiation, mice were contained in a 50 mL lead cylinder with air holes. Respective to the gamma and neutron irradiation, groups of 5 mice were given: 0, 0.25, 0.50, 0.75, 1.0 Gy. The gamma irradiation and neutron irradiation were at a dose rate of 114 mGy/min and 128 mGy/min, respectively. Table 1 outlines the irradiation group assignments.

	0 Gy	0.25 Gy	0.50 Gy	0.75 Gy	1.0 Gy
Gamma	5 mice; 10 samples	5 mice; 10 samples	5 mice; 10 samples	5 mice; 10 samples	5 mice; 10 samples
Neutron	5 mice; 10 samples	5 mice; 10 samples	5 mice; 10 samples	5 mice; 10 samples	5 mice; 10 samples

Table 1. Irradiation Group Allocation

2.4 Blood Sample Preparation and Cytometric Analysis

Approximately 44 hours after each group was irradiated, peripheral blood samples were obtained via cardiac puncture. Two blood samples were acquired from each mouse, and all blood samples were coded to prevent bias during analysis. The processing of the blood samples was in accordance with the procedures for a three-color flow cytometer as outlined in the MicroFlow® Mouse Micronucleus Analysis Kit (Litron Laboratories, Rochester, NY).

To summarize the procedures, 180 µl of heparinized blood was added to 2 ml ultracold fixative and stored at -75 ^{o}C to -80^{o}C until analysis. Before the cytometric analysis, the samples were washed and incubated simultaneously with RNase A, anti-CD71-FITC, and anti-CD61-PE (antiplatelet antibody) for approximately 45 minutes. Immediately prior to running the samples, the samples were resuspended in a prescribed amount of staining solution containing PI to obtain an appropriate flow rate on the cytometer. Before analyzing blood samples, the flow cytometer photomultiplier tube and compensation were calibrated using a malaria biostandard that mimics the signal of MN-RET [11]. The samples were analyzed on a Beckman Coulter EPICS XL flow cytometer (Beckman Coulter, Fullerton, CA). The anti-CD71-FITC, anti-CD61-PE and PI fluorescence signals were detected in the FL-1, FL-2, and FL-3 channels, respectively. Twenty thousand RET were analyzed per blood sample. To measure the cytotoxicity, the %RET was recorded. To measure the genotoxicity, the %MN-RET was recorded.

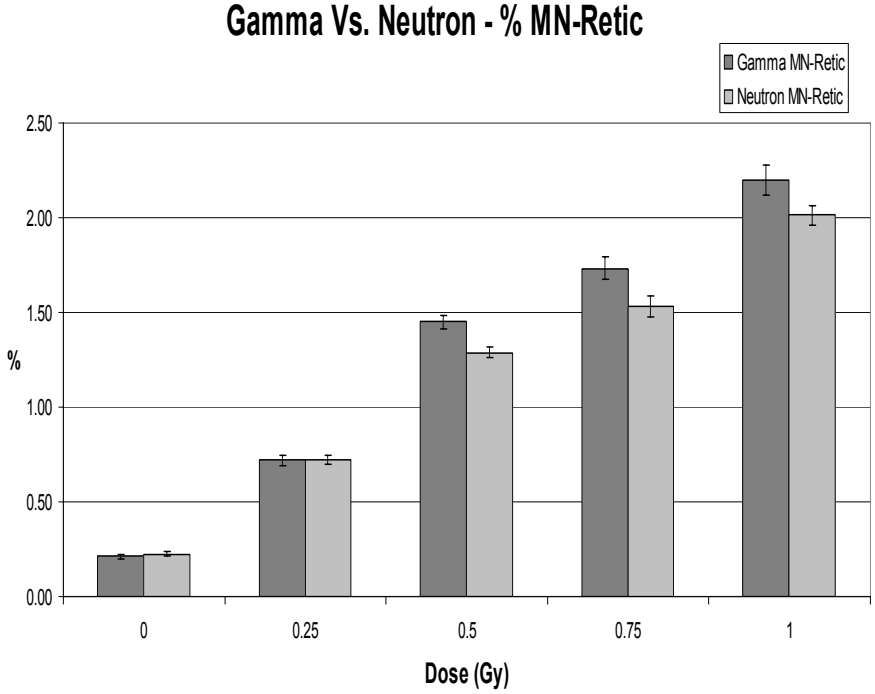
2.5 Statistics

The data values were represented as the mean of the 10 samples for each irradiation group. Error bars were calculated as the standard error of the mean. The Student's paired *t* test was used to calculate p-values between groups, and a one way ANOVA was used to determine overall significance between gamma and neutron irradiation vs. %RET and %MN-RET. Results were considered significant when $p<0.05$, two tailed.

3. Results

3.1 Dose Response

When analyzing the %MN-RET compared to dose, there is a significant dose response for gamma and neutron irradiation ($p <0.001$). Figure 1 shows the positive correlation between %MN-RET and dose from both forms of radiation. In regards to %RET, there is a negative correlation between %RET and dose from both forms of radiation (refer to Figure 2).



F**igure 1**: Comparison of %MN-RET vs. dose for gamma and neutron irradiation. A significant positive dose response for %MN-RET is evident, regardless of radiation quality. At the 0.5, 0.75, 1.0 Gy levels, gamma radiation induces significantly ($p<0.05$) more %MN-RET than neutron radiation.

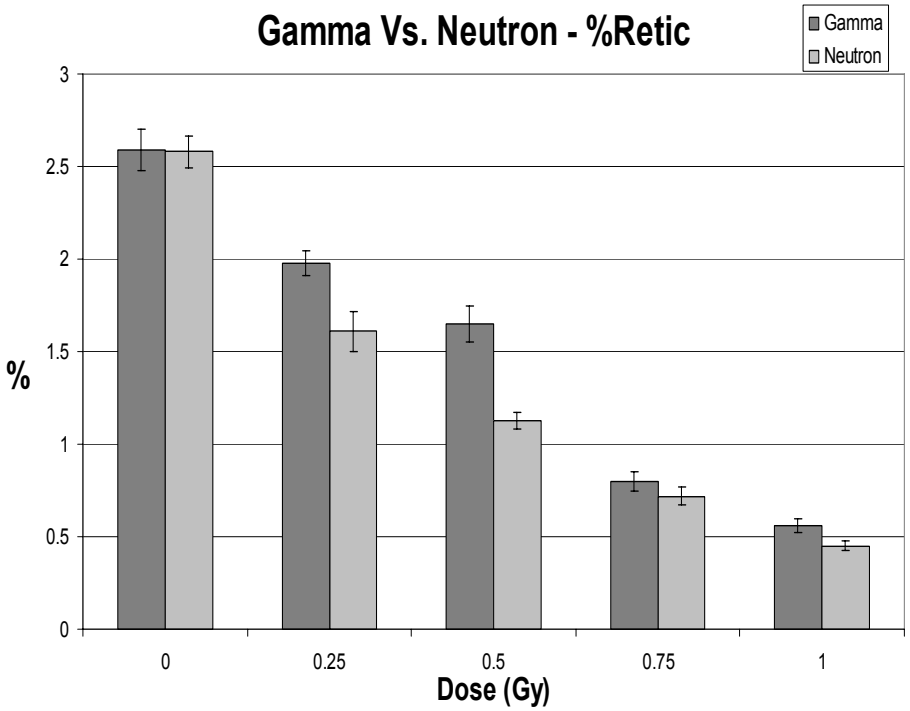


Figure 2: Comparison of %RET vs. dose for gamma and neutron irradiation. A significant negative dose response for %RET is evident, regardless of radiation quality. At the 0.25, 0.5, 1.0 Gy levels, gamma radiation induces significantly ($p<0.05$) more %RET than neutron radiation.

3.2 Effects of gamma radiation vs. Neutron radiation

When comparing the radiation quality and %MN-RET, At the 0.5, 0.75, 1.0 Gy levels, gamma radiation induces significantly ($p<0.05$) more %MN-RET than neutron radiation. When comparing the radiation quality and %RET, at the 0.25, 0.5, 1.0 Gy levels, gamma radiation induces significantly ($p<0.05$) more %RET than neutron radiation.

3.3 Effects of %MN-RET and %RET

Examining gamma irradiation, there is a significant ($P<0.001$) inverse correlation between %MN-RET and %RET (refer to figure 3). The same significant ($P<0.001$) inverse relationship is true for neutron irradiation (refer to figure 4). For both neutron and gamma irradiation, there is no evidence of %MN-RET or %RET leveling off for doses of 1 Gy and under.

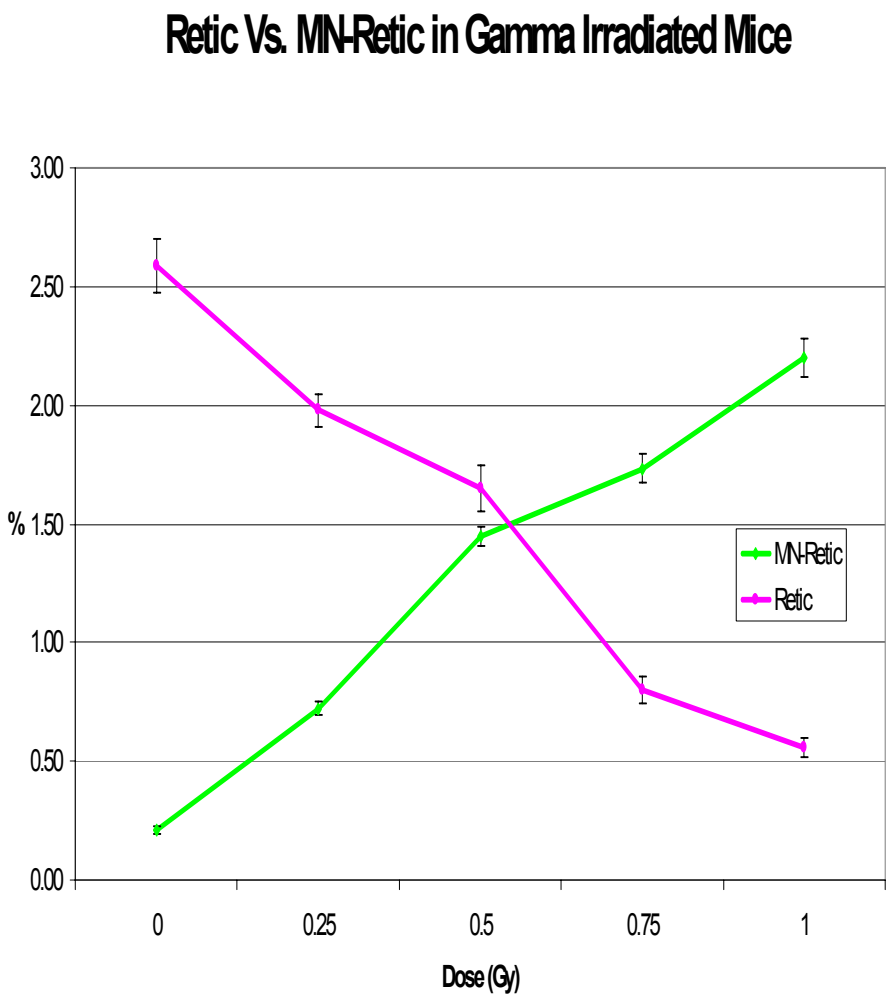


Figure 3: Comparison of %RET vs. %MN-RET in gamma irradiation, there is a significant inverse relationship. %MN-RET levels surpass %RET levels at around 0.6 Gy.

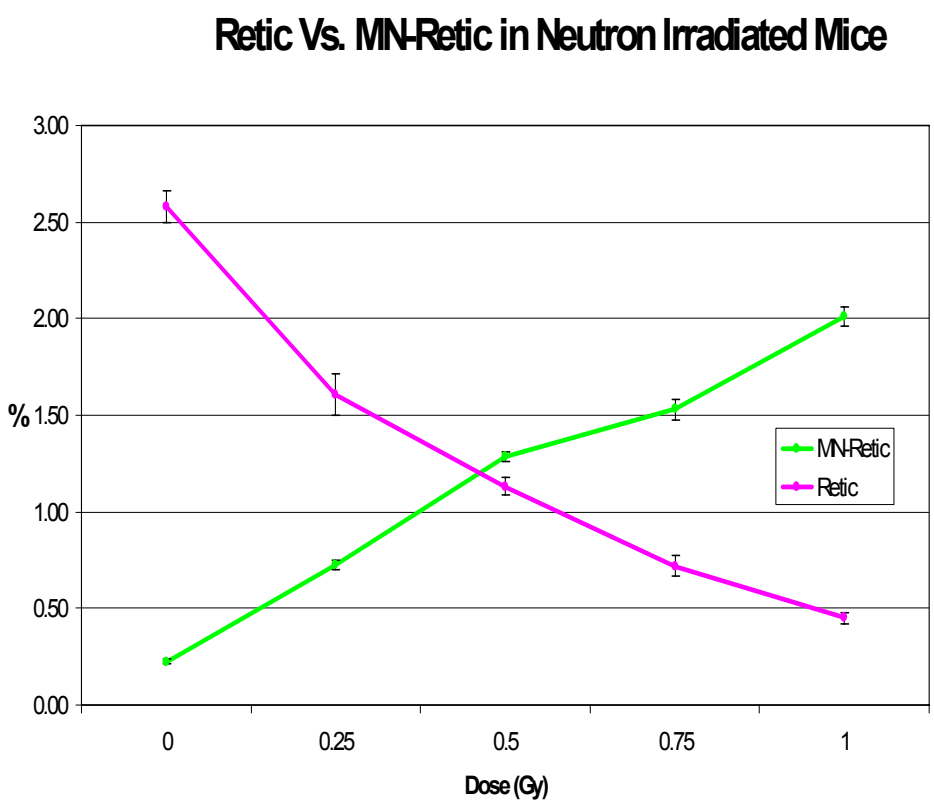


Figure 4: Comparison of %RET vs. %MN-RET in neutron irradiation, there is a significant inverse relationship. %MN-RET levels surpass %RET levels at around 0.45 Gy.

4. Discussion

The flow cytometry-based MN-RET assay proves to be a rapid and reliable alternative to gold standard cytogenetic damage assays via microscopy, such as dicentric and cytokinesis-blocked micronuclei assays [8]. At low doses of radiation, the MN-RET assay demonstrates high sensitivity as the %MN-RET and %RET end points exhibited consistency in the low dose responses. The MN-RET assay also demonstrates high reproducibility as supported by the low standard error values for all the experimental groups (refer to table 2 & 3). The present study results confirm the validity of the FCM-based MN-RET assay in determining induced cytogenetic damage via gamma and neutron irradiation. The consistency of the results supports the idea that the automation of MN-RET assay dose removes the interindividual variability that is often seen with results from subjective microscopy-based assays [5]. Furthermore, the automation of the MN-RET assay allows high throughput capability which is essential in large population triage.

In regards to cytotoxicity and genotoxicity induced by gamma and neutron radiation, the results appear to contradict previous research [6]. Having a higher RBE than gamma radiation for chromosomal aberrations [7], it is expected that neutron radiation would generate a higher %MN-RET. However, the results from this study show otherwise. Neutron radiation significantly ($p<0.05$) induced less %MN-RET than gamma radiation at 0.5, 0.75, 1.0 Gy levels. A possible explanation to the latter finding is that gamma radiation with a lower RBE than neutron radiation may be causing

DNA damage such as single-strand breaks or even double-strand breaks, but not sufficient to induce apoptosis, thus allowing more MN-RET to form than neutron radiation. To further support this explanation, the study results show that gamma radiation treatment induced more %RET than neutron radiation treatment. Neutron irradiation may be too damaging to the cells, thus, more cells undergo apoptosis than surviving through with DNA aberrations resulting in MN-RET formation. Further research is needed, however, to confirm that apoptosis in RET precursor and progenitor populations is occurring at higher frequencies in neutron radiation treatments than gamma radiation treatments.

GAMMA

	% MN RET		% RET	
Dose (Gy)	Mean	SE	Mean	SE
0	0.21	0.01	2.59	0.11
0.25	0.72	0.03	1.98	0.07
0.5	1.45	0.04	1.65	0.09
0.75	1.73	0.06	0.80	0.05
1	2.20	0.08	0.56	0.04

Table 2: Data for Gamma irradiation with respect to %MN-RET and %RET.

From the present study results, it can be asserted that in young RET populations, neutron radiation is more cytotoxic than gamma radiation as supported by the lower %RET. However, gamma radiation may be more genotoxic than neutron radiation as supported by the higher %RET. Despite conflicting results of gamma and neutron radiation when considering their well-accepted RBE, it must be noted that RBE values are dependant on many factors including radiation quality (LET), radiation dose, number of dose fractions, dose rate, the biologic system, and the endpoint [8]. For instance, there is research that has shown a higher RBE value for gamma radiation than neutron radiation in biologic systems such as the central nervous system [9]. In addition, there is research that shows equal RBE values for gamma and neutron radiation in human lymphocytes when the endpoint is apoptosi [10].

NEUTRON

	% MN RET		% RET	
Dose (Gy)	Mean	SE	Mean	SE
0	0.23	0.01	2.58	0.09
0.25	0.72	0.02	1.61	0.11
0.5	1.29	0.03	1.13	0.04
0.75	1.53	0.06	0.72	0.05
1	2.01	0.05	0.45	0.03

Table 3: Data for Neutron irradiation with respect to %MN-RET and %RET.

Irregardless of the contradicting results with previous research, the results observed in this study are valuable in that they demonstrate various dose-response properties associated with low doses (<1 Gy) of gamma and neutron radiation using the MN-RET endpoint in this particular mouse model. Given the trends observed and the consistency of the present research results, the possibility of developing an efficient and feasible way to triage large populations based on their radiation exposure in the event of a radiological disaster is ever-so promising.

5. Acknowledgments

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