ORIGINAL ARTICLE

Towards Establishing Capacity for Biological Dosimetry at Ghana Atomic Energy Commission

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ABSTRACT

The aim of this study was not only to obtain basic technical prerequisites for the establishment of capacity of biological dosimetry at the Ghana Atomic Energy Commission (GAEC) but also to stimulate interest in biological dosimetry research in Ghana and Sub-Saharan Africa. Peripheral blood from four healthy donors was exposed to different doses (0–6 Gy) of gamma rays from a radiotherapy machine and lymphocytes were subsequently stimulated, cultured, and processed according to standard protocols for 48–50 h. Processed cells were analyzed for the frequencies of dicentric and centric ring chromosomes. Radiation dose delivered to the experimental model was verified using GafChromic® EBT films in parallel experiments. Basic technical prerequisites for the establishment of capacity of biological dosimetry in the GAEC have been realized and expertise in the dicentric chromosome assay consolidated. We successfully obtained preliminary cytogenetic data for a dose-response relationship of the irradiated blood lymphocytes. The data strongly indicate the existence of significant linear (α) and quadratic (β) components and are consistent with those published for the production of chromosome aberrations in comparable absorbed dose ranges.

Key words: Biological dosimetry, dicentric chromosome aberration, dose-response curve, Ghana Atomic Energy Commission

Introduction

The high likelihood of a nuclear terrorist attack is one of the most immediate and extreme treats to global security.[1] In accident scenarios, physical dosimetry measurements may be unavailable or ambiguous, yet physicians would require dose estimates or physical dose reconstruction to complement other clinical diagnoses to prescribe appropriate medical care and investigate the long-term health effects.[2]

Timely and accurate biological dose estimates for victims caught in the crossfire of such disasters is critical. Bender and Gooch[3] proposed the assessment of the frequency dicentric chromosomes (DCs) in peripheral blood lymphocytes of exposed individuals for the detection of human radiation exposure. The conventional dicentric chromosome assay (DCA) which is sufficiently radiation-specific with reproducible dose-response relationship and low background frequency has since been accepted as a biomarker to assess human exposure to ionizing radiation and generally considered as the "gold standard" of the biodosimetric methods.[4]

Since the terrorist attacks on the World Trade Center on September 11, 2001, countries have taken proactive measures to establish competence in biological dosimetry even when nuclear activities are rarely in vogue.[5] Among others, biodosimetric information obtained from this postradiation event would help prevent overcrowding of treatment locations and facilitate dose-dependent treatment decisions and reassure minimally exposed victims. In the long-term, information gathered from biological dosimetry for low-dose exposures could assist epidemiological investigations such as cancer risk assessments and/or long-term disease risks.[6,7]

Reports have described wide inconsistencies in dicentric yields per gray and background chromosome aberration frequencies

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among several laboratories.[4,8] Hence, every biodosimetry laboratory is tasked to develop in-house calibration curves for each radiation dose and quality to minimize uncertainty in dose assessment.

In Ghana and most Sub-Saharan countries, biological dosimetry has remained a gray area. This brief report attempts to contextualize biological dosimetry in the framework of medical emergency preparedness, environmental radiation surveillance as well as set the pace for the commencement of biological dosimetry in Ghana. In addition, it serves as a vehicle to stimulate collaborative research work in biological dosimetry in Sub-Saharan Africa and encourage an Africa biodosimetry network.

Materials and Methods

The dose determination with GafChromic EBT® radiochromic films

The GafChromic EBT film dosimeter was calibrated as described elsewhere.[9-12] Briefly, cut pieces of GafChromic EBT dosimetry film were made watertight by sealing in thin polythene films. A series of the pieces of film was sandwiched between solid water phantoms (30 cm × 30 cm × 25 cm), irradiated for different durations at ambient temperature and pressure (field size = 10 cm × 10 cm; source to surface distance (SSD) = 100 cm; gantry angle = 0°; depth = 5.0 cm) to doses of 1, 2, 4, 6, and 8 Gy. Irradiated films and unirradiated controls were digitized with a flatbed document scanner (HP Scanjet G3110 Photo Scanner) and their mean optical densities analyzed using a standard densitometer (X-Rite Model 301). Net optical density (NOD) was obtained by subtracting the optical density of background of unirradiated controls from the optical density of irradiated films. A graph of dose against NOD was plotted using GraphPad Prism (GraphPad Software, San Diego, CA, USA) to obtain the standard calibration curve and calibration equation. The calibration curve was interpolated to convert optical density to absorbed dose.

Blood samples

This study was approved by RAMSRI-ERC (Ethics Approval Number RAMSRI-ERC 09/02/15). Blood samples (10 ml each) were drawn from four healthy volunteers (two males and two females), aged between 25 and 42 years, into sodium heparin tubes and mixed well. The heparinized blood was then aseptically split into sterile tubes (1.1 ml/tube) and coded 0, 1, 2, 4, and 6 Gy.

Irradiation of blood lymphocytes and confirmation of absorbed dose

Coded tubes containing whole blood from donors were irradiated to graded doses of γ-rays (0–6.0 Gy) at room temperature, using a Theratron Equinox (Atomic Energy of Canada Ltd., Ottawa, Canada) at a dose rate of 0.888 Gy/min. To ensure uniformity of radiation absorbed dose, samples were placed at a SSD of 100 cm. To verify that the physical dose delivered was the dose reaching the target volume, pieces of GafChromic EBT2 dosimetry film of similar dimensions were introduced into experimental tubes as before (field size = 10 cm × 10 cm; SSD = 100 cm; Gantry angle = 0°; depth = 5.0 cm) to give doses of 1, 2, 4 and 6 Gy as the experimental samples. Films were digitized using an HP Scanjet G3110 Photo Scanner and their mean optical densities analyzed using a standard densitometer (X-Rite Model 301). Unirradiated pieces of film were used to provide a background measurement of the optical density and NOD obtained by subtracting the optical density of background radiation from the optical density of irradiated film.

Lymphocyte culture and analysis

Five hundred microliters of each unirradiated (control) and irradiated blood sample was cultured in duplicate in 4.5 ml of RPMI 1640 growth medium in T25 cell culture flasks which were also coded: 0, 1, 2, 4, and 6 Gy. The RPMI medium was supplemented with Hepes, sodium bicarbonate, 15% fetal bovine serum, 1% L-glutamine (2 mM), antibiotics (penicillin: 100 U/ml; streptomycin 100 μg/ml), and 6 μg/ml phytohemagglutinin A end concentration. Cells were incubated at 37°C in an environment of 5% CO_2 and 95% air for 48–50 h. Following this period, 50 μl of colcemid was added to each culture tube and the incubation continued for a further 3 h, upon which the cell cultures were terminated for subsequent processing of dicentric chromosome yield.

Dicentric chromosomal aberration assay

At the end of the culture period, cell cultures were terminated and transferred to new 15 ml conical polypropylene tubes and centrifuged at 1300 RPM for 7 min after which the supernatant was carefully discarded. Five milliliters of hypotonic solution (0.075 M KCl) was added dropwise with constant mixing, followed by centrifugation at 1300 RPM for 7 min. The supernatant was gently decanted and the cell pellet gently and thoroughly resuspended in 7 ml freshly prepared fixative (methanol:glacial acetic acid, in a ratio 3:1, vol/vol). The cells were centrifuged again at 1300 RPM for 7 min and the supernatant completely drained. Cells were washed 2x or 3x in the fixative (or repeatedly until the pellet was white). After the last wash, the fixative was discarded leaving 500–1000 μl above the pelleted cells.

Coded slides corresponding to each sample were made slightly damp by carefully running a damp filter paper over the working surface. The fixed cells were resuspended by gentle vortexing of the tubes. A volume of 15–20 μl of the cell suspension was gently dropped at the center of each coded slide. For each sample, five slides were prepared. The slides were air dried at room temperature for at least 24 h. Slides were stained in 3% Giemsa (1.5 ml Giemsa in 50 ml 1x Sorenson's buffer) for 9–10 min. The slides were then carefully rinsed under a gentle stream of running tap water and allowed to dry after which coverslips were mounted, using Entellan mounting medium (Merck, Darmstadt, Germany).

Metaphase chromosomes were assessed for radiation damage for each radiation absorbed dose point as described elsewhere.[4,13] Manual scoring of metaphases of at least 50 cells was scored for dicentrics and centric rings using a light microscope. For this study, only dicentric and centric rings were scored in metaphase spreads that contained 46 chromosomes.

Statistical analysis

Dose-response calibration curves were constructed with GraphPad Prism (GraphPad Software, San Diego, CA, USA) computer program. Standard equations were used to fit nonlinear relationships. Data were calculated as the means (±standard error) from three independent experiments. For each experiment and data point, 3 replicates were assessed.

Results and Discussion

The authors are unaware of any previous biological dosimetry study conducted in Ghana. This study aims at establishing a competent biodosimetry laboratory capable of performing cytogenetic analysis for possible use in mass casualty radiation accidents, emergency preparedness, or environmental radiation surveillance. In the wake of an advancing nuclear era and increasing threats of radiological terrorism, studies of this nature are highly warranted and the construction of in-house dose-response curves for various radiation doses and qualities is very important. The most validated biodosimetry techniques for dose estimation are the DCA, widely accepted as the gold standard, and lately, the cytokinesis-block micronucleus assay.[4]

To build competence for the establishment of a cytogenetic biodosimetry laboratory in the Ghana Atomic Energy Commission (GAEC), it is important to have the basic equipment in place and in proper working condition, and to build a solid team comprising medical officers, scientists, and technologists capable of drawing blood, processing samples in a professional manner, and technically capable of scoring chromosome aberrations (for this study) or any biological endpoint of interest reproducibly. Furthermore, a very high throughput means of assessing the radiation exposure based on these biological endpoints is critical. The onus will then be on this team to generate standard calibration dose-response curves for different types and energies of radiation. As the DCA is considered as the "gold standard," we concentrated on building the biodosimetry facility by first establishing competence in DCA cytogenetic analysis.

In terms of equipment, our laboratory has basic items for biodosimetry such as a laminar flow hood (for processing samples), CO_2 incubator (to maintain the study environment for cells), a table top centrifuge, an air oven (sometimes used for culturing purposes), and a light microscope (for endpoint scoring). These are very basic and quite obsolete and would need upgrading or complete replacement to bring the center up to speed.

Regarding the availability of human resources, two technologists (BSc) and six scientists (one PhD, three MPhils and two MScs) are capable of setting up lymphocyte cultures, processing and scoring dicentric and centric ring chromosomes. None of the officers has spent time undertaking rigorous training in an accredited cytogenetic biodosimetry laboratory which is an essential prerequisite for obtaining accreditation for the establishment of a recognized biodosimetry laboratory. In addition, we have a senior medical physicist (PhD candidate), an oncologist, and a radiologist with expert knowledge in dosimetry and treatment planning as part of our team. Moreover, our main collaborator in South Africa is an eminent radiobiologist and an expert in data analysis making our team replete.

In this report, dicentric chromosome aberrations were evaluated for five dose points: 0, 1, 2, 4, and 6 Gy on human peripheral blood lymphocytes irradiated *in vitro* with gamma rays. The main types of structural chromosome aberrations elaborated were dicentrics, acentric fragments, and centric rings; for this purpose, dicentric and centric ring chromosomes in first-division metaphase spreads containing 46 chromosomes were scored. The physical radiation doses delivered to the lymphocytes were confirmed by first establishing a standard curve with GafChromic® radiochromic films irradiated to 0–8 Gy where the films were sandwiched between a water phantom at a depth of 5 cm, field size of 10 cm × 10 cm, and at an SSD of 100 cm. Subsequently, the film samples were digitized with a flatbed document scanner (HP Scanjet G3110 Photo Scanner), the NOD for each film sample calculated, and the calibration and experimental curves obtained by fitting the analytical forms:

$$\text{Standard fit: } \text{NOD} = 0.78[1 - e^{(-0.12\times\text{dose})}] \quad (1)$$

$$\text{Experimental fit: } \text{NOD} = 0.57[1 - e^{(-0.15\times\text{dose})}] \quad (2)$$

These were subsequently used to evaluate experimental absorbed doses and the results obtained are displayed in Figure 1. From the Figure 1, it is observed that to a very fair approximation delivered doses matched standard and experimental doses. When the given doses were compared with standard and experimental doses using the paired *t*-test, the means were not significantly different (R^2 = 0.9999; P = 0.4202), demonstrating a good precision of delivery.

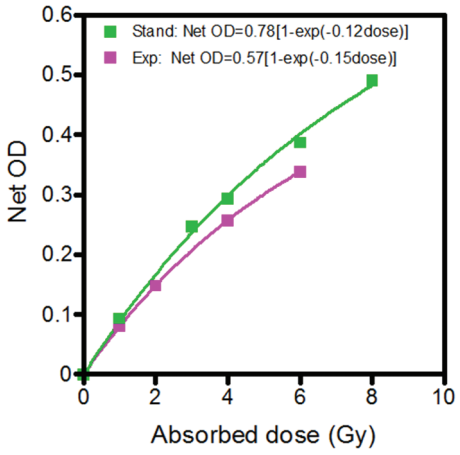

Figure 1: Fitted dose-response curves for GafChromic EBT films, using an HP Scanjet G3110 Photo Scanner. The standard curve (green line) shows radiation dose delivered to the watertight GafChromic films sandwiched between water phantoms at a depth of 5 cm and irradiated at a gantry angle of 0°C. The actual dose received by lymphocytes (experimental curve) was established upon irradiation of GafChromic films in a parallel experiment to mimic the real experiment. Maximum dose delivered to experimental setup was 6.0 Gy while that standard setup was 8.0 Gy

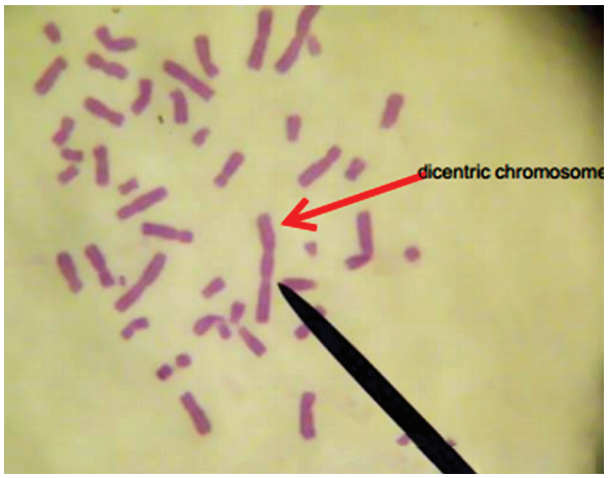

Figure 2: A representative metaphase spread from an irradiated sample: an abnormal metaphase spread showing radiation damage, in the form of a dicentric (pointed by arrow) to at least one chromosome

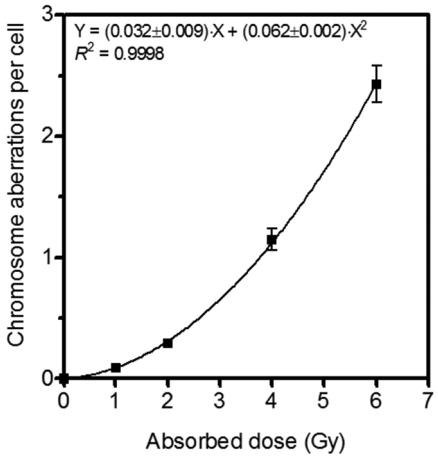

Figure 3: The yields of dicentric chromosomes in peripheral blood lymphocytes as a function of radiation absorbed dose. Symbols and error bars represent the mean and standard error of the mean of triplicate samples, respectively

The number of dicentrics per cell increased as a function of radiation dose. Figure 2 represents chromosomes of cells exposed to γ-rays. The arrowed chromosome depicts a DC. The determined frequency of radiation-induced chromosomal aberration is plotted against radiation absorbed dose in Figure 3.

The data strongly indicate that the frequency of chromosome aberration in lymphocytes of the studied group of donors (Y) can be described as a function of absorbed dose (X) using the second order polynomial of the form: $Y = \alpha X + \beta X^2$. A strong positive correlation ($R^2 = 0.9998$) between dicentric yield and absorbed dose and the curve coefficients are displayed on the graph. The values of α (0.032 ± 0.009) and β (0.062 ± 0.002) coefficients are in close agreement with the findings of Savage.[14] The volume of data captured in this study is quite limited given that this is just a brief report. In future, when more data are imputed, the values may alter slightly and background chromosome aberration may become evident and significant. In this study, impaired cell proliferation was realized after high-dose exposures (≥4.0 Gy). Thus, the volume of cell preparations dropped on the slides was doubled to ensure that an adequate number of cells were available for scoring.

The established dose-response curve remains to be validated wherein blood samples from human volunteers will be irradiated to known doses of ^{60}Co γ-radiation and assessed from the curve.

Conclusion

Basic technical prerequisites for the establishment of capacity in biological dosimetry in the GAEC have been realized and expertise in the DCA assay consolidated. Preliminary cytogenetic data revealed a clear dose-response relationship between radiation dose and chromosome aberration. A strong existence of significant linear (α) and quadratic (β) components consistent with published studies for the production of chromosome aberrations in comparable absorbed dose ranges is demonstrated. To increase statistical strength for the low dose region (0.2–2.5 Gy), the team intends to focus future work on adding more dose points within the relevant region.

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Conflicts of interest

There are no conflicts of interest.

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