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Evolution of the Health Canada astronaut biodosimetry program with a view toward international harmonization

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ABSTRACT

Biodosimetry of astronaut lymphocyte samples, taken prior to- and post-flight, provides an important *in vivo* measurement of radiation-induced damage incurred during space flight which can be included in the medical records of the astronauts. Health Canada has been developing their astronaut biodosimetry program since 2007 and since then has analyzed data from 7 astronauts.

While multiple cytogenetic endpoints may be analyzed for the astronauts, the Fluorescent *in situ* hybridization (FISH) assay is considered to be key for detecting long-lasting stable damage. It is believed that this long-lasting damage is most likely to lead to an increased risk to the health of the astronauts during long-term flights (lasting 6 months or more).

The complexity of damage that results from protracted, non-homogeneous radiation exposure, like that found in the space environment, requires a detailed scoring schematic to capture as much information as possible. To that end, this paper outlines the efforts to harmonize the manner in which Health Canada's FISH data are recorded to better facilitate the comparison of results with other international biodosimetry programs.

1. Introduction

Cytogenetic biodosimetry has provided dose estimates for accidental overexposures for over 50 years [1]. These methods have been applied to astronauts since the early 1990's [2–4] by collecting lymphocyte samples prior to- and post-flight to provide an important *in vivo* measurement of radiation-induced damage incurred during space flight [4,5]. Astronauts are exposed to a wide array of ionizing radiation while in space [6,7] and although physical dosimeters are present on-board, the spectrum varies and it is difficult to account for the biological effects of attenuation of the body, especially in the presence of other situational stress [8]. Additionally, research has shown that the cellular damage incurred in space is not simply additive after repeated time in space, and that more complex mechanisms underlie the biological response [9]. Biodosimetry allows all of these factors to be taken into account and for Canadian and European astronauts, biodosimetry testing is a medical requirement as specified in the International Space Station (ISS) Medical Evaluation Document [10].

Several different cytogenetic assays are available for biodosimetry, two of the most widely used being the Dicentric Chromosome Assay (DCA) and stable translocations as measured by Fluorescent *in situ* hybridization (FISH) [1,11]. The DCA scores the number of dicentric chromosomes in Giemsa-stained metaphase spreads and correlates the amount of damage to a given dose. However, uncertainty arises due to the rate of loss of dicentrics over time [12,13] as they are less likely to survive mitosis. The estimated half-life of lymphocytes containing dicentrics varies greatly, and there is evidence that it ranges from months to approximately 3 years [6,12,14]. In comparison, the FISH assay is a technique which uses specific probes to paint individual chromosomes, allowing for easy visualization and scoring of chromosome translocations, providing a way to score unstable damage such as dicentrics, as well as long-lasting stable damage not visible with the conventional staining of the DCA [15]. The limits of detection for the DCA and FISH assays are approximately 0.1 Gy [16] and 0.3 Gy respectively [17] when no pre-exposure sample is available. When a pre-exposure sample is available, the limit of detection of the FISH assay is comparable to the DCA [13]. The types of damage which can be visualized using FISH probes can be quite varied and complex [18]. Tucker et al. [15] established a descriptive system known as the Protocol for Aberration Identification and Nomenclature Terminology

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(PAINT). The system allows for a flexible scoring schematic that can be used to describe unlimited arrangements of translocations without reference to the mechanism of aberration formation. Stable translocations can persist in blood for many years, as they are more likely to survive mitosis, unlike the shorter lived unstable damage [19,20]. Additionally, any stable damage that might occur in stem cells would continue to be replicated as the stem cells divide. Therefore, when scoring a sample, it is important to differentiate between stable and unstable cells and to note any possible clone cells. As well, while the background rate of dicentrics in a healthy blood sample is very low, typically 1 in 1000, the background rate of translocations can be slightly higher and increases with age. It is not uncommon to find up to 10 stable translocations in 1000 scored cells [13,17,20,21].

To overcome the issue of increased background rate of translocations when performing biodosimetry for astronauts, it is common practice to generate a calibration curve from the astronaut's own blood prior to a mission. This allows the rate of translocations post-flight to be measured relative to individual background levels [6,22].

While earlier missions to space spanned a few weeks and radiation induced translocations were difficult to detect above background, astronauts are now spending months on the International Space Station (ISS) and are, therefore, receiving higher doses. Equivalent dose rate estimates as measured by physical dosimetry, vary from approximately 0.2–0.5 mSv per day [23] and extensive work by Cucinotta et al. [24] determined the average effective dose for a 6 month stay on the ISS to be approximately 72 mSv. These doses can cause levels of damage that are measurable by FISH and they will continue to increase as space agencies move to progressively longer space flights. The higher doses received from extended missions will be well within the FISH detection limit.

As the numbers of astronauts undertaking long missions on the ISS are low, it is important that the different laboratories and agencies performing biodosimetry harmonize their methods of data collection with a unified scoring schematic in order to be able to compare results. Between 2007 and 2015, Health Canada has analyzed samples from 7 astronauts. The methods described below were developed in consultation with the National Aeronautics and Space Administration (NASA) to ensure the collection of comparable data. Being able to compare results with other space agencies will provide improved data with which the risk of protracted exposures can be better understood. This paper describes the methods and schematics used by Health Canada for analysis of astronaut samples, with a view toward such a harmonization.

2. Material and methods

2.1. Sampling and shipping

Astronauts were sampled as required by the ISS Medical Evaluation Document [10]. For each astronaut, a venous blood sample was taken pre-flight and two more samples taken post-flight (after 1–2 weeks and 6 months–1 year). The pre-flight sample included three 6 mL lithium heparin (Li–He, Becton Dickinson (BD), Oakville, ON, Canada) Vacutainer® tubes and one 4 mL ethylenediaminetetraacetic acid (EDTA) Vacutainer® tube (BD). Post-flight samples included one 6 mL Li–He tube and one 4 mL EDTA tube. The extra volume in the pre-flight samples was used for generating a dose calibration curve (0–2 Gy) unique to each astronaut.

Samples were shipped according to appropriate Transportation of Dangerous Goods protocols and shipments included temperature control gel packs (conditioned to remain at room temperature) as well as a temperature logger and an OSL chip to monitor temperature changes and any radiation exposures, respectively, which may have occurred during shipping. Packages were express shipped overnight in order to be processed as quickly as possible. Control samples (sham and 2 Gy) were drawn from a volunteer in the laboratory at the same time as the scheduled astronaut blood draw and processed alongside the astronaut samples. All volunteer blood donors gave informed consent in a research protocol approved by Health Canada's Research Ethics Board.

2.2. Irradiations and sample culture

Whole blood samples, taken pre-flight, were exposed to *in vitro* doses ranging from 0 to 2 Gy in a cabinet X-ray chamber (X-RAD320, Precision X-ray, North Branford, CT, USA) at 250 kV, 12.5 mA, with a focus to surface distance (FSD) of 50 cm, 4.5 cm polystyrene build-up material and 2.5 mm Al + 0.13 mm Cu filtration. The field size was 10 cm × 10 cm at the point of measurement. The dose rate was 0.82 Gy/min, measured using a PTW TW30010 (PTW, Freiburg, Germany) ion chamber calibrated at a national primary standards laboratory (N_k = 48.3 mGy/nC at 250 kV). Post-irradiation, whole blood was incubated for 2 h on a rocker at 37 °C and 5% CO_2 to allow for cellular repair.

After the 2 h incubation, whole blood cultures were initiated as described by IAEA [1]. Briefly, whole blood was added, at a 1:10 ratio, to culture medium (RPMI 1640, Invitrogen, Burlington, ON, Canada) which was supplemented with heat inactivated 15% fetal bovine serum (Sigma–Aldrich, Oakville, ON, Canada), 2 mM L-glutamine-penicillin-streptomycin (Sigma–Aldrich), 5 μg/mL 5-bromo-2′-deoxyuridine (BrDU) (Sigma–Aldrich) and stimulated with 2% phytohemagglutinin (PHA, Invitrogen). Cultures were incubated at 37 °C and 5% CO_2 for 48 h. After 44 h, Colcemid (Invitrogen) was added to a final culture concentration of 0.1 μg/mL to block the lymphocytes in first metaphase. Cells were harvested after treatment with 0.075 M potassium chloride and fixed with fresh Carnoy's fixative (3:1 methanol:acetic acid) and placed in a −20 °C freezer for a minimum of 30 min prior to slide preparation.

2.3. Slide preparation and staining

FISH was performed using the following probes: chromosome 1 (Texas Red spectrum, red signal), chromosome 2 (Fluorescein Isothiocyanate (FITC) spectrum, green signal) and chromosome 4 (Texas Red and FITC spectra, resulting in a yellow signal). Staining was carried out according to the standard protocol provided by the probe manufacturer (Cytocell 1,2,4 Direct Probe, Rainbow Scientific; Windsor CT, USA).

Fixed samples were washed twice with fresh Carnoy's fixative at room temperature and the pellet resuspended. 15 μL of cell suspension was dropped on each slide and slides were checked for adequate concentration and chromosome spreading.

The slides were aged at 37 °C (between 24 and 72 h) before being soaked in 2x saline-sodium citrate (SSC) buffer (pH 7.0, Sigma–Aldrich) for 2 min, then dehydrated in a series of ethanol baths (70%, 80% and 100%), each for 2 min and placed on a 37 °C slide warmer. 18.5 μL of probe (warmed to room temperature) was placed on the slide and cover-slipped with a 22 × 40 mm glass coverslip. To prevent dehydration, the edges of the coverslip were sealed with rubber cement glue and allowed to dry completely. For denaturation of the chromosomes, the slides were heated to 75 °C for 2 min and then allowed to hybridize at 37 °C for 24 h.

Post-hybridization, the glue and coverslips were carefully removed and the slides washed in 0.4x SSC buffer (Sigma–Aldrich) at 72 °C and pH 7.0 for 2 min. The slides were drained and washed in 2x SSC, 0.05% Tween-20, pH 7.0 (Sigma–Aldrich) at room temperature for 30 s. Again the slides were drained and 20 μL of DAPI