MONITORING THE GENETIC HEALTH OF PERSONS IN GOIÂNIA ACCIDENTALLY EXPOSED TO IONIZING RADIATION FROM CAESIUM-137

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Abstract

MONITORING THE GENETIC HEALTH OF PERSONS IN GOIANIA ACCIDENTALLY EXPOSED TO IONIZING RADIATION FROM CAESIUM-137.

This work describes the long term genetic monitoring of the Goiânia population exposed to ionizing radiation from ^{137}Cs, using cytogenetic and molecular endpoints. Cytogenetically, micronucleus frequencies differentiated groups exposed to different levels of radiation. Two molecular methods were employed: 1) the hprt clonal assay, involving in vitro selection of 6-thioguanine-resistant hprt mutant clones which were characterized at the molecular level using RT-PCR and genomic analysis. Ionizing radiation exposure initially elevated hprt mutation frequency which gradually diminished, so that no significant increase was observed four and a half years after original exposure. The spectrum of hprt mutations recovered from ten individuals exposed to relatively high doses of radiation revealed a fourfold increase in the frequency of A:T → G:C transitions. The increase is consistent with the effects of ionizing radiation in prokaryotes and lower eukaryotes. Additionally, a twofold increase in the frequency of deletions was observed which may reflect radiation induced DNA strand breakage; 2) determination of microsatellite instability using fluorescent PCR and genomic DNA from mononuclear cells. The frequency distributions of somatic microsatellite alterations in exposed and non-exposed populations were not different. Our assay lacked sensitivity to discriminate between spontaneous and induced microsatellite instability and therefore, is not suitable for population monitoring. Finally, we estimated the risk associated with radiation exposure for the exposed Goiânia population. The estimated genetic risk of dominant disorders in the first post-exposure generation was increased nearly twenty-fourfold. The risk of carcinogenesis was increased by a factor of 1.5.

1. INTRODUCTION

"The great tragedy of Science – the slaying of a beautiful hypothesis by an ugly fact."

T.H. Huxley

Environmental mutagens and carcinogens have been suspected as the cause of many human diseases. To date, a number of short term approaches utilizing both eukaryote and prokaryote models are used to identify potential mutagens and carcinogens as well as to assess their impact on the environment. The results obtained in such studies are used to eliminate such agents from the environment or to develop strategies to minimize inevitable exposures.

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Over the last five decades, intensive study has been conducted to understand the biological effects of exposure to ionizing radiation in humans at the molecular, cellular, and organismic levels. Despite these efforts, little is known about the mechanisms responsible, perhaps because of the complex nature of ionizing radiation and its complex interactions with biological matter. Nevertheless, exposure to ionizing radiation has been established as a hazard to human health by contributing to the mutational load and increasing the cancer incidence in exposed populations. This knowledge has clearly caused widespread concern about the biological effects of radiation exposure.

As biotechnological approaches have become available, they have allowed more detailed studies and a better understanding of the biological effects of exposure to ionizing radiation. In particular, it is now possible to determine the nature of mutations in an individual at the DNA sequence level. Such technological investigation allows for a more precise interpretation of the molecular events underlying radiation-induced mutations and contributes to defining the mutational specificity of ionizing radiation.

On 13 September, 1987 in Goiânia (Brazil), a chain of unfortunate events led to a serious radiological accident that became known in the scientific community as simply the "Goiânia accident". During the two weeks following the accident, 249 people received substantial exposure to ionizing radiation from ^{137}Cs, resulting in four fatalities. Individual dose estimates ranged from near zero up to 7 Gy. In addition, personnel taxed with the subsequent patient care and cleanup were also exposed to low doses of ionizing radiation. This unfortunate accident, however, has provided a unique opportunity to study the nature of ionizing radiation-induced mutations in humans.

Moreover, the medical actions and radiation protection measures undertaken by the emergency teams resulted in the definition of a well characterized exposed group which was ideal for a follow-up study. The considerable efforts made to determine individual exposures, the continuing cytogenetic follow-up, and the detailed monitoring of the clinical health of the exposed individuals, maximize the potential value of our study.

The subjects had received a combination of external beam irradiation, skin contamination and internal contamination. Several methods were used to estimate the dose received, including: (1) internal dosimetry — bioassay and whole body monitoring; (2) cytogenetic dosimetry — the estimation of doses by chromosomal aberrations analysis; (3) external dosimetry — dose estimates from reconstruction and on the basis of radiation effects. The details on how dose estimates were determined are contained in the IAEA report on the accident [1].

Biodosimetric results obtained one year after the accident, using the methods of somatic 'null' mutation at the glycophoryn A locus and chromosome translocations detected by in situ hybridization (these assays were not readily available at the time of the accident), are in general agreement with the results obtained immediately after the accident using dicentric chromosome aberrations [2].

The overall proposal of the follow-up study was the monitoring of the genetic health of the individuals exposed to ionizing radiation during the Goiânia accident. The study involved the application of biotechnology to investigate the mutagenic effects of ionizing radiation at both cytogenetic and molecular levels, as well as to determine the consequences of radiation to the exposed and general populations by estimating the risk of carcinogenesis and genetic harm associated with radiation exposure. The applied strategy involved the use of methodologies and a combination of several techniques, such as the growth of T-lymphocytes under selective and non-selective conditions, polymerase chain reaction (PCR), automated DNA sequencing, and non-sequencing applications of an automated DNA sequencer, to name just a few.

The general objective of the long term study was to determine the DNA damage caused by ionizing radiation, as well as to investigate the nature of mutation in the exposed population in Goiânia. The blood samples were collected at yearly intervals from both exposed and control populations from Goiânia, on a voluntary basis. The control group consisted of unexposed individuals selected from unexposed neighbours, family members, and workers of the Fundação Leide das Neves Ferreira[1]. Although this accident provides a rare opportunity to investigate in vivo the radiation-induced mutations in people, it lacks the refinement of experimental studies conducted under the lens of previously designed protocols. In addition, accidental exposures generally involve complex populations displaying the normal human heterogeneity which impose some limitations. This is especially true considering both the limited number of individuals available and the reduced availability of samples. We must, therefore, emphasize that some of these limitations are unavoidable and hence were present during the development of this study.

2. OVERALL DISCUSSION

Exposure to ionizing radiation is a fact as old as life itself. However, the perception of the potential danger of radiation has become a common worry of the modern world. Human beings and all other living creatures have constantly been exposed to natural sources of radiation, including cosmic radiation and external and internal irradiation from radionuclides within the organism. Moreover, the development of radiological and nuclear technologies has, to some extent, contributed to human exposure due to these man-made sources. Over the past century, few nuclear issues have commanded as much public and scientific attention as those which relate to radiation. Public awareness of the risks associated with radiation has increased, perhaps in response to the contribution from man-made radiation. Yet a gap still remains between scientific documentation and public perception of exposure to radiation.

Prevention, detection, assessment, and risk estimation are important procedures to evaluate the impact that occupational, accidental, therapeutic, or environmental radiation exposures may impose on human health. A clear understanding of both biological and physical consequences of such exposure provides the bases for critical decisions concerning both the handling of radioactive material and the development of monitoring and follow-up approaches. The first line of defence is prevention, including both occupational and accidental exposures. Prevention could be readily achieved by using physical methods to monitor involved personnel and by reinforcing the safety guidelines and the laws relating to the management of radioactive material.

Scientific knowledge relating to radiation effects is continuously expanding and advances in modern biology should make it possible to determine the sensitivity to ionizing radiation of an individual person. This would contribute to minimizing individual exposure to medical radiation, identifying those individuals who require increased clinical surveillance for cancer, and finally determining whether radiation is indeed the cause of some cancers and some genetic disorder.

Harm to the environment and ultimately to humans caused by radiation is almost entirely associated with nuclear accidents or the use of nuclear weapons. Such unfortunate conditions provide unique opportunities to investigate the potential biological effects of radiation exposure on human health. The nuclear weapons legacy comprises the dropping of two atomic bombs in August 1945 on Hiroshima and Nagasaki. Approximately 600 000 inhabitants of these two cities were affected by this terrible historical episode. Of that number

[1] A foundation established by the state of Goiás to assist the victims of the accident.

only about 100 000 individuals survived. The Hiroshima and Nagasaki residents comprise the largest group of individuals who have been followed medically for over 40 years. The list of nuclear accidents has been increasing. Two major accidents worldwide were the Chernobyl accident in April 1986, the largest nuclear accident in the Eastern world, and the Goiânia radiological accident in September 1987, the largest in the Western world. Both events were characterized by communication gaps between the population, political decision-makers, the media, and medical experts.

The Goiânia radiological accident provided us the opportunity to understand some of the biological effects of exposure to ionizing radiation imposed on a population. The people exposed have expressed great concern for their future health, including particular anxiety regarding the potential for the genetic harm to their offspring and their own risk of cancer development. From 1990 until the present, we have been monitoring the genetic health of some of the individuals exposed to ionizing radiation in the Goiânia accident, with doses ranging from 0.1 to 7 Gy. Our approach was first to investigate and determine possible exposure by using the micronucleus assay as the cytogenetic endpoint. Secondly, we carried out studies at the molecular level, using the *hprt* gene as the molecular endpoint. We conducted a longitudinal study to determine both the level and the fate of *hprt* mutation frequency in T-lymphocytes of individuals exposed to high doses of ^{137}Cs as well as the nature of mutations induced in that population. We then performed a comparative study with the spontaneous spectrum of mutations at the *hprt* gene, as well as compared our spectrum of mutations with both the Goiânia low dose group spectrum and the A-bomb survivor spectrum. Again, at the molecular level we investigated the possible effect of ionizing radiation on the induction of microsatellite alterations using MNCs from exposed and non-exposed individuals. Finally, we used both the micronucleus and the *hprt* data to estimate the genetic harm and the risk of carcinogenesis in the Goiânia population.

Using the micronucleus assay we performed an investigative study on a random sample of 276 individuals. Even one year after exposure, we demonstrated that the assay had strong predictive utility for high dose exposures, as long as comparison with control individuals is used. Unfortunately, the micronucleus assay cannot detect low levels (< 0.2 Gy) of ionizing radiation. Additionally, prediction of individual dosage (true dosimetry) is not possible. We conclude, however, that the micronucleus assay is a useful biological dosimeter for human populations even if blood samples are taken a year after exposure to ionizing radiation. While the human lymphocyte micronucleus assay cannot replace detailed chromosomal analysis for precise estimations of radiation doses, it can be used as a quick predictive model of exposure for screening purposes.

When we performed the longitudinal study on the ten individuals exposed to high levels of ionizing radiation with doses ranging from 1 to 7 Gy, two major findings were revealed: 1) the *hprt* mutant frequency was higher in those exposed to high doses of ^{137}Cs ionizing radiation during the Goiânia radiological accident than in the control group obtained from the same population; 2) the *hprt* mutant frequency of the exposed individuals decreased gradually over time, showing that the *hprt* T-cell assay is not suitable for the study of long term past exposure because of its poor long term memory. Four and one-half years after exposure, the mutant frequencies of the exposed group were indistinguishable from the background frequencies of the Brazilian control group and mutant frequencies reported by others [3, 4]. In addition, we found an age related increase in mutant frequency of 3.3% per year which is consistent with a 3% per year increase in the *hprt* mutant frequencies in T-cells of five control populations [3]. We also estimated a 2.1-year half life for those T-cells having *hprt* mutations, which is directly responsible for the decrease in mutant frequency with time, following exposure. This study

demonstrated that the *hprt* assay has low sensitivity and, therefore, is of limited value for long term monitoring.

In order to establish possible differences between our spectral sample comprised of mutants obtained from 10 individuals exposed to high doses of ionizing radiation, we used the hypergeometric test in 2-by-6 tables. We compared our HD-1991 spectrum of *hprt* mutations based on cDNA changes with the AB spectrum, LD-1990, and lastly with the SS spectral sample, and found no significant difference ($p \geq 0.05$). However, Chi-square analysis, comparing our HD-1991 spectrum and the SS, revealed that there was a potential difference between these two groups centered on base substitutions. A significant increase (close to fourfold) in the frequency of A:T → G:C mutations was found in the exposed group. Peculiarly, A:T → G:C transitions at position 278 (5/57) were recovered in five of the exposed individuals. This particular transition has not yet been reported in any of the spontaneous *hprt* data sets and may even be representative of past exposure to ionizing radiation. It is noteworthy that A:T sites seem to be favoured over G:C pairs as targets in our study population, a trend which was shown to be statistically significant. Damaged A:T base pairs in our HD-1991 comprised some 80% (18/23) of base substitutions, as opposed to 35% (34/98) among the spontaneous mutants. This increase in the frequency of A:T → G:C transition in our exposed population may reflect mutation due to a particular radiation-induced lesion, most likely due to mispairing of radiation-induced thymine glycol with a guanine. This finding is consistent with experiments in bacteria [5–8], bacteriophage [8, 9], and lower eukaryotes [10], where detailed molecular analysis had been undertaken. Moreover, it has been reported that A:T base pairs are more susceptible to radiation and oxidation-induced mutagenic damage than G:C pairs in a test strain (TA102) of *Salmonella* [6]. A similar sensitivity for A:T sites has been reported for ionizing radiation in *E. coli* [7]. These observations are consistent with the shift from G:C → A:T sites reported here.

In our study, 30% (27/90) of the deletions shared the common feature of not being readily explained by slippage events which may reflect the direct effects of ionizing radiation-induced DNA strand breakage. This class of deletion was represented in our spectral sample at a frequency close to twofold greater than the frequency observed in the spontaneous database.

We further investigated the potential induction of microsatellite instability by exposure to ionizing radiation. We quantified the frequency of new alleles arising somatically in mononuclear cells of peripheral blood. Using fluorescent PCR and the ALF DNA sequencer (Pharmacia), combined with the ALF2SMA® analysis, we demonstrated 5.3% and 4.5% of microsatellite alterations in non-exposed and exposed individuals, respectively. Our findings suggest that mismatch repair deficiency (or poor proficiency) is compatible with cell development. Our current approach lacked the sensitivity to discriminate between spontaneous and induced microsatellite instability and it is, therefore, not suitable for monitoring. Nonetheless, our findings opened a window for the potential use of somatic microsatellite instability arising in a normal population. This could become an effective tool in identifying individuals with somatic mutations in DNA repair genes, as well as in identifying clonal microsatellite alterations in body fluid which have important implications in cancer detection and diagnosis.

Finally, we examined the possible genetic risk imposed by radiation exposure in the Goiânia population. We examined both the genetic harm and the risk of carcinogenesis for that discrete population. We attempted to use our experimental data on micronucleus and *hprt* mutant frequency to obtain some sense of the risk involved in human exposures. While this has provided a useful lesson, our small population size makes the interpretation of the results difficult. Interpretation is further complicated by the nature of the assumptions and the

limited data upon which estimates are made. Nevertheless, our estimates can be considered a reasonable indicator of the level of genetic damage for that population. We obtained risk estimates of a close to twenty-fourfold increase in dominant disorders in the first post-exposure generation of the directly exposed population. No detectable increase was found in the population at large. The risk of carcinogenesis in the directly exposed population was found to be increased by a factor in the range of 1.4 to 1.5. The added risk of carcinogenesis may be genuine and it is consistent with the number of individuals in the exposed population who had developed cancer by 1995. However, as it is rarely possible to categorically attribute a specific cancer to ionizing radiation, we can only conclude that it appears that the probability of carcinogenesis is indeed higher in the exposed population.

Keeping in mind that risk estimation encompasses complex calculations and is based upon a number of uncertainties and assumptions, we support Sobels' observations that human somatic mutation can be used as a good indicator of ionizing radiation in humans [11, 12]. Therefore, extrapolations to the gametic level would be possible if the relationship between somatic and gametic mutations in mouse and humans were better understood. This would require the assumption that mice and humans are affected the same way, which at this time, however, we cannot state unequivocally.

We attained the long term objective of this project for which we applied and developed methodologies for the purpose of monitoring a selected subset of individuals who, unfortunately, were exposed to ionizing radiation. Throughout the course of this work, the patient population remained accessible and co-operative, contributing to helping future generations. They also silently and patiently hoped that we would bring some good news that would minimize their suffering and anxiety about the future and would reduce the burden of being victims. Perhaps we do bring good news at last. The general conclusions were that we reported a decrease in mutant frequencies, a small risk of carcinogenesis, and an almost irrelevant risk for the next generation. The conclusions are all strongly supported by rigorous scientific work and literature. The results may even bring some degree of well-being back to the exposed population of Goiânia, especially since no health consequences have yet been directly linked to radiation exposure. Post accident traumatic stress and recurrences of primary radio-lesions have been the major causes of concern for the severely exposed individuals. Our conclusions should mostly reiterate the need for the continuation of long term follow-up protocols and clinical surveillance, with increased epidemiological support. Moreover, it should be emphasized that clinical follow-up and monitoring studies must be performed completely independently of socio-political programmes.

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AUTHORS' NOTE

As a result of this study a series of scientific papers have been published containing all the relevant tables of results and figures. Copies can be obtained upon request at the following addresses: (1) Centre for Environmental Health – Department of Biology, University of Victoria, PO Box 3020, Victoria, B.C. Canada, V8W 3N5; (2) Departmento de

Ciências Biológicas e Biomédicas, Universidade Católica de Goiás, Av. Universitária, 1440 – S. Universitário, Cx. Postal 86, 74605-010, Goiânia, Brazil; or (3) by sending electronic messages to this effect to acruz@uvic.ca, acruz@netline.com.br, bwglick@uvic.ca.

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