BIOLOGICAL DOSIMETRY OF ABSORBED RADIATION BASED ON THE FREQUENCIES OF CHROMOSOMAL ABERRATIONS IN HUMAN LYMPHOCYTES

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Introduction

In the investigation of radiation accidents, it is important to estimate the dose absorbed by exposed persons in order to plan their therapy For this task information such as magnitude of dose received, as external or internal radiation exposure, or partial or whole body irradiation are necessary For example following exposure to high doses (> 5 Gy) of low LET radiation the victim may need bone marrow transplantation and in case of partial body exposure to high doses the victim may be isolated and kept under very hygienic conditions in order to allow repopulation from surviving stem cells in the bone marrow

Methods to estimate radiation doses

Usually in accidents the victims do not carry a physical dosemeter unless the accident happens in a nuclear facility. In the absence of any physical dosimetry one has to resort to biological methods. Even if physical dosimetric information is available, it is better to confirm this estimate with a biological dosimetry. Currently the fully developed biological indicator for exposure to ionizing radiation is the study of chromosomal aberrations in peripheral blood lymphocytes. Other techniques such as ESR measurements of tooth enamel are available but not yet well validated.

Chromosomal Aberrations

Most of the circulating lymphocytes are in a presynthetic stage of the cell cycle (G0) in the body Immediately following radiation exposure the chromosomal aberrations are formed. Lymphocytes with aberrations can circulate in the body for months to years. Lymphocytes are usually stimulated in culture for about 48 h with a mitogen whereby they enter the cell cycle and undergo mitoses. The mitotic cells are arrested by colcemid and fixed. Air dried preparations are stained and chromosomal aberrations are analyzed in the first mitosis in culture (IAEA, 1986)

Types of chromosomal aberrations

Exposure of blood lymphocytes at the G0 stage leads to chromosome type of aberrations such as dicentrics, rings, translocations, inversions and acentric fragments. Except for translocations and inversions all aberration types are unstable and will be gradually eliminated during cell division.

Dose-response curves

In general the frequencies of dicentrics are used to estimate absorbed doses. The frequencies of dicentrics induced by radiation in vitro and in vivo appears to be very similar for a given dose and therefore dose-response curves generated from in vitro studies can be used with confidence to estimate doses of in vivo exposure The frequency of exchange aberrations (dicentrics and translocations) increase in a linear quadratic manner following acute low LET radiation [Y = k + (D + (D2] With high LET radiation in a linear dose response is observed [Y = k + (D] For chronic low LET exposure the yield is mostly linear with the dose .

Dose estimates

The aberrations among lymphocytes after low LET radiation follow a Poisson distribution, while aberrations induced by high LET radiation as well as partial body radiation are overdispersed among the lymphocytes From the distribution of aberrations among the cells and the nature of the accident one can use an appropriate calibration curve to estimate the dose. Thus, in principle it is possible to estimate doses following exposure to acute as well as chronic low LET radiation, high LET radiation, partial body radiation and a mixture of these The calibration curves should be based on scoring large numbers of cells for each dose point, especially at the low dose range (up to 1 Gy) using at least 2 different blood donors, preferably a male and a female. The spontaneous frequency of dicentrics in lymphocytes is about 1 in 1000 cells (IARC, 1986) In a suspected exposure case, a minimum of 500 to 1000 cells are to be scored and if the frequencies are less than the spontaneous one the person can be considered to be unexposed. There are several factors which can influence the accuracy of the dose estimate. These include (a) the calibration curve, (b) the number of cells scored and © the number of aberrations observed and the distribution of aberrations. Based on these factors a dose estimate is made by using the maximum likelyhood method (Papworth, 1975). The sensitivity of this technique is limited up to 0.1 - 0 2 Gy and very much depends on the number of cells scored in each case.

For making dose-estimates the blood sample should be drawn within a day following exposure. For delayed sampling a correction factor can be applied assuming that the average lifetime of lymphocytes is about 3 years (IAEA, 1986). However, the estimates for the average lifespan of lymphocytes vary considerably In a recent study on the radiation victims of the Goiânia accident, the average half-life based on the disappearance of lymphocytes carrying dicentrics was estimated to be between 110 and 470 days depending on the radiation dose received (Ramalho et al., 1995). Cytogenetic studies of atom bomb victims from Hiroshima

and Nagasaki indicate that a long lived sub-population of lymphocytes can have a lifespan of more than 50 years This makes the dose estimates based on dicentric frequencies from delayed sampling, using a correction factor for the average half-life of lymphocytes not very reliable

Unlike dicentric chromosomes which are lost in the body with time, balanced translocations are expected to persist Thus one can use the frequencies of translocations for dose estimates immediately after irradiation as well as for past exposure. Translocations can be detected by using chromosome banding techniques but these are very laborious Recently the Fluorescence In Situ Hybridization Technique (FISH) using chromosome specific DNA libraries has been successfully employed to detect translocations (Natarajan et al., 1991; Pinkel et al., 1986). Though the frequencies of translocations and dicentrics are expected to be induced at equal frequencies, in vitro radiation studies show that translocations are induced at a much higher rate than dicentrics. This is mainly due to the contribution of incomplete and complex translocations in addition to balanced reciprocal translocations (Bauchinger et al., 1993, Natarajan et al., 1992).

In view of the persistant nature of translocations it has been suggested that their frequencies can be used to estimate doses of past exposures For example, translocation frequencies in atom bomb survivers appear to be similar to the frequencies induced in vitro by different doses of radiation (Lucas et al., 1992a) However, other studies on radiation victims from Goiânia and Chernobyl indicate that the frequencies of translocations at later sampling times may not reflect the frequencies induced at the time of the accident (Granath et al , 1996, Natarajan et al., 1997, Salassidis et al., 1995a, b).

The Goiânia accident

In this accident more than 100 individuals were contaminated with 137Cs in September, 1987 The 137Cs was from a radiotherapy unit which was stolen, broken into pieces and distributed to several individuals Immediately after discovery of the accident, blood samples were collected and processed for chromosome analysis at the Institute of Radiation Protection and Dosimetry, Rio de Janeiro. The frequencies of dicentrics and their distribution were determined. Since the exposure in this accident was chronic in nature, a dose response curve which had been generated earlier based on low dose rate (-rays was used to estimate the doses. The doses of 129 individuals were estimated which varied from 0 - 7 Gy From the distribution of aberrations, individuals with whole body and partial body irradiation were identified (Ramalho et al., 1988) Later on a new dose response curve was generated with protracted irradition and based on that curve the dose estimates were recalculated (Ramalho, 1992). The comparison of the individual dose estimates and the history of the accident indicated a very close correlation between highly exposed individuals and high doses (IAEA, 1988), demonstrating the validity of using frequencies of dicentrics to estimate absorbed radiation dose in accidents.

Retrospective dosimetry based on translocation frequencies

Earlier studies using banding techniques indicated that the translocation frequencies remain stable over many years following therapeutic radiation of patients with Ankylosing Spondylitis (Buckton, 1983) However, the initial translocation frequencies were not determined in these patients. In addition to the studies on the atom bomb victims, retrospective dosimetry has been used for a radiation worker accidentally exposed to tritium (Lucas et al , 1992b), cancer patients undergoing radiation therapy (Gebhard et al , 1996) and Tschernobyl victims (Salassidis et al., 1995a, b) These data suggest that with few exceptions the frequencies of translocations remain constant for several years In all these cases the initial frequencies of dicentrics or translocations were not determined Victims of the Goiânia accident form an unique cohort of a radiation exposed population for which data on initial frequencies of translocations are available (Natarajan et al., 1997). In a follow-up study the frequencies of translocations in 24 of these victims who recieved estimated doeses between 0.1 to 5 3 Gy were determined using FISH techniques Several coctails of chromosomes covering about 80 % of the genome were used and a total of 71,245 metaphases were analyzed. The frequencies of translocations in the group of individuals who received more than 1 Gy were lower than the initial dicentric frequencies. The difference was about a factor of 3 In individuals who received doses less than 1 Gy the translocation frequencies were also lower than the initial dicentric frequencies and in individuals who received 0 4 Gy or less the frequencies of translocations were similar or more than the initial frequencies of dicentrics. At these low doses to get reliable dose estimates, it is obvious that one should score 10,000 to 20,000 cells per individual, making it a very difficult task to perform . A similar conclusion was also reached in the follow up studies of the Estonian clean up workers in the Chernobyl accident (Granath et al., 1996). In such cases at best one can discriminate between the exposed and control group without attempting to estimate individual doses

In a mouse model we studied the question as to whether and with what characteristics dicentrics and translocations decline with time. Mice were irradiated with 2 Gy X-rays and the frequencies of dicentrics and translocations were determined in splenocytes 0 to 112 days after irradiation (Natarajan et al., 1996) As can be seen from Fig. 3, the translocations disappear much slower and with other characteristics than dicentrics

References

Bauchinger M, Schmid E, Zitzelsberger H, Braselmann H, Nahrstedt U, Radiation-induced chromosomal aberrations analysed by two colour fluorescence in situ hybridization with composite whole chromosome-specific DNA probes and a pancentromeric DNA probe. Int J. Radiat Biol , 64, 179-184, 1993.

Buckton KE , Chromosome aberrations in patients treated with X-irradiation for ankylosing spondylitis In: Ishihara T and Sasaki MS (Eds.) Radiation-Induced Chromosome Damage in Man Alan R. Liss, New York, pp 491-511, 1983

Gebhart E, Neubauer S, Schmitt G, Birkenhake S, Dunst J, Use of three-color chromosome in situ suppression technique for detection of past radiation exposure. Radiat Res , 145, 47-52, 1996.

Granath F, Darroudi F, Auvinen A, Ehrenberg L, Hakulinen T, Natarajan AT, Rahu M, Rytomaa T, Tekkel M, Veidebaum T, Retrospective dose estimates in Estonian Chernobyl clean-up workers by means of FISH. Mutation Res , 369, 7-12, 1996

IAEA, Biological Dosimetry: Chromosomal Aberration Analysis for Dose Assessment, Technical Reports Series No 260, International Atomic Energy Agency, Vienna, 1986

IAEA , The radiological accident in Goiânia. IAEA STI/PUB/815, Vienna, 1988

Lucas JN, Awa A, Straume T, Poggensee M, Kodama Y, Nakano M, Ohtaki K, Weiner H-UG, Pinkel D, Gray J, Littlefield G, Rapid translocation frequency analysis in humans decades after exposure to ionizing radiation. Int J Radiat. Biol., 62, 53-63, 1992a.

Lucas JN, Poggensee M, Straume T, The persistence of chromosome translocations in a radiation worker accidentally exposed to tritium. Cytogenet. Cell Genet , 60, 255-256, 1992b.

Natarajan AT, Vyas RC, Wiegant J, Curado MP, A cytogenetic follow-up study of the victims of a radiation acident in Goiânia (Brazil) Mutation Res., 247, 103-111, 1991

Natarajan AT, Vyas RC, Darroudi F, Vermeulen S, Frequencies of X-ray induced chromosome translocations in human peripheral lymphocytes as detected by in situ hybridization using chromosome-specific DNA libraries Int. J. Radiat. Biol., 61, 199-203, 1992.

Natarajan AT, Boei JJWA, Darroudi F, Van Diemen PCM, Dulout F, Hande MP, Ramalho AT, Current cytogenetic methods for detecting exposure and effects of mutagens and carcinogens. Environ. Health Persp., 104, 445-448, 1996

Natarajan AT, Santos SJ, Darroudi F, Hadjidikova V, Vermeulen S, Chatterjee S, van der Berg M, Grigorova M, Sakamoto-Hojo ET, Granath F, Ramalho AT, Curado MP, 137Cesium - induced chromosome analyzed by Fluorescence In Situ Hybridization. Eight years follow up of the Goiânia radiation accident victims Mutation Res, in press, 1997.

Papworth DG, Curve fitting by maximum likelihood. Radiat Bot., 15, 127-140, 1975.

Pinkel D, Straume T, Gray JW, Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization. Proc. Natl. Acad Sci. (USA) 83, 2934-2938, 1986.

Ramalho AT, Nascimento ACH, Natarajan AT , Dose assessments by cytogenetic analysis in the Goiânia (Brazil) radiation acident. Radiat. Prot Dosim , 25, 97-100, 1988.

Ramalho AT, PhD Thesis, "Subsidios a tecnica de dosimetria citogenetica gerados a partir da analise de resultados obtidos com o acidente radiologico de Goiânia". Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, 1992

Salassidis K, Schmid E, Peter RU, Braselmann H, Bauchinger M, Dicentric and translocation analysis for retrospective dose estimation in humans exposed to ionizing radiation during Chernobyl nuclear power plant accident Mutation Res., 311, 39-48, 1995a.

Salassidis K, Georgiadou-Schumacher V, Braselmann H, Muller P, Peter RU, Bauchinger M, Chromosome painting in highly irradiated Chernobly victims: a follow-up study to evaluate the stability of symmetrical translocations and the influence of clonal aberrations for retrospective dose estimation. Int. J. Radiat: Biol., 68, 257-262, 1995b.