Biological Dosimetry in cases of accidental and occupational exposure to ionising radiation: State of the art

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1. Abstract: The importance of detecting risks at the low doses (<5cGy) and low dose rates in radiation protection is well recognised. Similar issues surround the acquisition of knowledge on common genetic factors that might determine inter-individual differences in low dose cancer risk. These aspects are of continuing importance in respect of social/economic policy relating to the industrial and medical uses of ionising radiation, and for risk assessment among people occupationally are being exposed to low and/or high LET radiation, such as astronauts, pilots, stewardess and nuclear power plant workers, as well as victims of radiation accidents. In order to emphasis on the acquisition of fundamental knowledge and the development of low dose as well as high dose risk models for low LET radiation (i.e. gamma-rays and X-rays) and high LET radiation (i.e. heavy ions, alpha-particles, neutrons), several biological assays were developed and attempts were made to investigate formation of radiation induced chromosome aberrations and induction of genomic instability in human following acute and/or chronic exposure. Newly obtained data indicate that (a) Premature chromosome condensation assay is a unique method to be used for immediate dose assessment at low does (5≥Gy) as well as high doses (≥3 Gy), and can accurately discriminate between whole- and partial-body exposure. Therefore, this is the method of choice for biodosimetry in cases of mass casualties and accidental over-exposure to high doses of ionising radiation (b) Fluorescence in situ hybridization (FISH) technique using chromosome, chromosome-arm, chromosome region, centromere and telomere specific DNA libraries has improved the resolution of detecting all classes of radiation induced chromosomal inter- and intra-changes. Consequently, has increased significantly the accuracy and detection limit of biological dosimetry. FISH-based translocation assay has the potential to assess biodosimetry in cases of accidental as well as occupational exposures to ionising radiation, either immediately following exposure, and in particular, retrospectively by defining accumulative effects to red bone marrows. (c) Further modification of FISH assay by either combining chromosome, centromeres and telomeres specific probes or the M-FISH assay revealed distinct finger-prints, such as insertions and complex translocations for high LET radiation in comparison to low LET radiation. Keywords- Ionising radiation, Biological dosimetry, Chromosomal aberrations, PCC, FISH-based translocation, I. The importance of dose assessment in human: Considering the notion that chromosomal instability is the hallmark of cancer, it is essential to develop and validate different biological assays for dose-assessment in cases of occupational and accidental over-exposure to ionising radiation of different qualities. High doses of ionizing radiation clearly produce deleterious consequences in human, including, but not exclusively, cancer induction. Therefore, it is essential to estimate chromosomal

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aberration incidences in cases of high exposures ($\geq$ 3 Gy acute). These sets of information on dose assessment can assist in the planning of therapy and in altering physicians to likely deterministic health consequences that could arise in the following weeks and years. At low-doses and low dose rates of radiation ($<$ 1 Gy) the situation is much less clear. In animal studies at a dose rate up to 1mGy/day and a total dose of 400 mGy of 137Cs-gamma-rays no effect on shortening the length of life span, as well as on incidences of neoplasms in mice was evident (1,2). In human by analysing the Techa river cohort, recently, epidemiological data reported that, risks associated with low dose rate exposure for solid cancer incidence are not less than those seen following acute exposures such as were received by atomic bomb survivors (3,4). Moreover, there showed no significant non-linearity in the dose response. Consequently, these data revealed that the risk assessments of low dose radiation are of great importance in relation to occupational exposure (chronic).

II. Biological assays used for dose assessment immediately following exposure to ionising radiation: Different cytogenetic assays such as dicentric in a metaphase, micronucleus (MN) in a binucleated cell, premature chromosome condensation (PCC) in a interphase cell are being used in human peripheral blood lymphocytes as gold-standards to define frequency of spontaneously occurring chromosomal alterations in unexposed population as well as to estimate the absorbed dose in a short period of time following a radiation accident (5). In general dicentric analysis is being used for radiation dose assessment immediately following exposure. However, difficulties in dose estimation arise for the past exposure (acute), as well as chronic exposure (occupational) due to a decline of cells containing unstable chromosome aberrations (i.e. dicentrics). Fluorescence in situ hybridization (FISH) - based translocations technique has opened new perspectives for rapid and precise detection of stable chromosome aberrations (5-7). The inherent stability of translocations over cell generations has enabled them to be used as a biodosemeter (5-8).

III. Requirement for assessment of Biological dosimetry: a. Generation of in vitro calibration curve (chronic and acute exposure) Conventionally, the frequency of dicentric has been employed as biological dosemeter, by using calibration curves that are generated by irradiating human blood samples in vitro. In general, the dose effect curves following an acute exposure for low LET radiation conform to the equation $Y = c + \alpha D + \beta D2$, where Y is the yield of chromosome aberrations, c is the background frequency, α and β are fitted coefficients of aberration production and D is the dose (in grays) (5). For dicentrics and translocations, irradiation with X-rays or gamma-rays following whole body exposure produces a Poisson distribution among the lymphocytes, whereas high LET radiation produces an over-dispersed distribution. In cases of chronic exposure to low LET radiation as well as chronic or acute exposure to high LET radiation, the frequency of chromosome aberration can be estimated using the equation $Y = C + \alpha D$ (5). b. Age and life style of donors In order to assess reliably dose of exposure (i.e. low doses and past exposure), it is of great importance to generate enough data for unexposed control population. The spontaneous frequency of di-

centrics and translocations in metaphases, micronuclei in binucleated cells, and premature chromosome condensation in interphase cells in human is estimated to be in the range of 1-4, 0-18, 2-35 and 0-2 per 1000 genome equivalent cells analysed respectively. Data available so far indicate, among population several variables, such as age, smoking habits, ethnics and/or occupational / environmental over-exposure, that may influence the frequency of spontaneously occurring chromosomal alterations. Among these age effect seems to be the most prominent one, in particular for MN and translocation in human lymphocytes (5, 9, 10) IV. Dose assessment immediately and Detection of total- and partial-body irradiation The efficacy of three cytogenetic methods (dicentric, MN and PCC) for assessment of the unirradiated fraction and the persistence of damage after total-body (TB) and partial-body (PB) irradiation in human lymphocytes (in vitro) was studied. Human lymphocytes were X-irradiated with life threatening doses of 5 and 8 Gy, and mixed in different proportions [(0% (means that 100% irradiated), 10%, 30%, 50%, 70%, 90% and 97%] with unirradiated lymphocytes of the same donor. PCC could accurately estimate the fraction of irradiated cells (independent of fractions irradiated). Unlikely dicentric and MN analyses consistently underestimate, namely due to cell killing and mitotic delay effects that are operating at high exposure levels. V. Retrospective dosimetry using FISH-based translocation assay Because translocations are appeared to be stable, one can use their frequencies to retrospectively estimate past radiation exposure. The idea of using chromosomal translocations for retrospective biological dosimetry has been initiated for many years in Japanese atomic bomb survivors. In the last decay it has become easy to identify translocations using FISH-technique. Consequently, a large number of studies were performed (using Goiania; Chernobyl, Istanbul and Mayak cohorts) to focus and unify the work, and results obtained (6-8) could give information on: 1. What should be painted? 2. to unifying scoring of translocations two nomenclatures were introduced to classify the different aberration types, PAINT and S&S systems, this issue shed light on what should be scored? 3. What are the control levels of translocations? 4. Do stable translocations persist with time after exposure? For retrospective biological dosimetry it has become usual to paint three of the larger pairs of chromosomes with a single or different colour and to highlight centromeres and/or telomeres with a different colour and to apply a counter-stain (6, 11). Recently, in order to detect all structural and numerical aberrations among human chromosomes a multi-colour FISH assay, so called Combined Binary Ratio labelling (COBRA), for simultaneous visualization of all human chromosomes in 23-24 different colours is developed (12). This technique is successfully applied to detect translocation in an accidental exposure (Istanbul) to 60Co gamma-ray. Furthermore, the suitability of MN, diecentric analysis for dose assessment immediately and retrospectively was studied. Though all three systems proved to be useful for immediate dose assessment, However, a rapid decline was found with MN and diecntric even within first 6 months after exposure. In contrast, the frequency of translocation remained constant for the entire period of three years that this analyses was performed. The outcomes revealed that for a reliable biological dosimetry using of FISH-based translocations, the

following parameters are of great importance and should be considered, as follows: Translocations in stable cells should be measured only in cells that contain the full complement of the painted material. Two-way and one-way translocations should be combined with equal weight. It is also worthy to note that in all radiation exposure incidences (accidentally or occupationally) studied (Goiania, Chernobyl and Mayak) the frequency of hyperploidy cells tends to increase with time (7). Using either COBRA M-FISH (12, 13) and triple colour FISH, it was found that translocations are persistent with time if measured in stable cells only. COBRA-MFISH was found to have a greater resolving power over partial labelling for the accurate detection of complex translocations and insertions. With high LET neutrons the frequencies of both were higher than those induced by X rays, and for insertion the relative proportions to the total frequencies of translocations remained constant. These data suggest insertions and complex-type translocations can be used as the 'signature' of high LET radiation. VI. Recommendation for future actions: Perspective area of further investigations in the field of biological consequences of Chernobyl accidents as well as in cases of occupational exposure (i.e. Mayak and Techa River cohorts) for human health seems to be further work on the radiation induced chromosomal aberrations, namely stable translocations not only in irradiated persons but in their progeny and so-called "bystander effect", as well as the evaluation of possible connection between the genome structure damages both stochastic (oncopathology) and non-stochastic (multifactorial pathology) radiation effects. Furthermore, state of the art technology such as M-FISH should be applied in order to detect different classes of induced translocations. However, it is also recommended to generate a battery of test systems and to join efforts between studies in biological dosimetry and epidemiology in order to elucidate on the role of induced chromosomal aberrations and cancer formation in cases of radiation over-exposure.

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REFERENCES 1. Tanaka S, Tanaka IB, et al. (2003) No lengthening of life span in mice continuously exposed to gamma rays at very low dose rates. Radiat Res. 160; 376-379. 2. Tanaka IB, Tanaka S, et al. (2007) Cause of death and neoplasia in mice continuously exposed to very low dose rates of gamma rays. Radiat Res. 167; 417-437. 3. Kristinina LY, Davis F, et al. (2007) Solid cancer incidence and low dose-rate radiation exposures in the Techa River cohort: 1956 2002. Int J Epidemiol. 36; 1038-1046. 4. PrestonDL, Cullings H, et al. (2008) Solid cancer incidences in atomic bomb survivors exposed in utero or as young children. J Natl Cancer Inst. 100; 428-436. 5. International Atomic Energy Agency (2001), Cytogenetic Analysis for radiation Dose Assessment: A Manual. Technical Report series No. 405). 6. Darroudi, F. (2000) Use of FISH translocations analysis for retrospective biological dosimetry: How stable are stable chromosome aberrations? Radiat Prot Dos. 88; 101-109. 7. Darroudi, F. and Natarajan, AT (2000) Application of FISH chromosome painting assay for dose reconstruction: State of the art and current views. Radiat Prot Dos. 88; 51-58. 8. Edwards AA, Lind-

holm C, et al. (2005) Review of translocations detected by FISH for retrospective Biological dosimetry applications. Radiat Prot Dos, 113; 396-402. Review. 9. Whitehouse CA, Edwards AA, et al. (2005) Translocation yields in peripheral blood lymphocytes from control populations. Int J Radiat Biol. 81; 139-145. 10. Sigurdson AJ, Ha M, et al. (2008) International study of factors affecting human chromosome translocations. Mut Res. 652; 112-121. 11. Fomina J, Darroudi F, et al. (2001) Accurate detection of true incomplete exchanges in human lymphocytes exposed to neutron radiation using chromosome painting in combination with a telomeric PNA probe. Int J Radiat Biol. 77; 1175-1183. 12. Darroudi F, Bezrookove V, et al. (2002) Insights into the sites of X-ray and neutron induced chromosomal aberrations in human lymphocytes using COBRA-MFISH. Radiat Prot Dos. 99; 189-192. 13. Bezrookove V, Smits R, et al. (2003) Premature chromosome condensation revisited: A novel chemical approach permits efficient cytogenetic analysis of cancers. Genes Chromosomes Cancer. 38; 177-186.