Optimisation and application of "state-of-the-art" cytogenetic techniques for determining genetic damage in the peripheral blood lymphocytes of individuals and populations occupationally or accidentally exposed to ionising radiation.

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Introduction

Epidemiological studies have shown that exposure to ionising radiation may induce malignant diseases in the exposed individual. A recent re-evaluation of the incidence of cancer and leukaemia in populations exposed to ionising radiation has resulted in an international recommendation (ICRP report 60 1991) to lower the dose limits for workers exposed occupationally to ionising radiation and for members of the public. Consequently, a systematic investigation of radiation-induced damage is of great importance for the population of radiation workers at risk in the nuclear industry and medical sector. Radiation accidents in foreign countries have pointed to the need for a reliable methodology, allowing quick and accurate measurement of the radiation burden received, not only for a restricted number of individuals but also for a sizeable fraction of the population.

Genetic damage plays a critical role in radiation-induced carcinogenesis. Therefore cytogenetic techniques are preferred for evaluating individual risk associated with occupational or accidental exposure to ionising radiation. The incidences of chromosomal aberrations and micronuclei in peripheral blood lymphocytes are known practical bio-indicators of radiation damage. The use of these cytogenetic techniques in biomonitoring radiation workers solves several specific problems inherent in physical dosimetry, such as impracticability in the case of partial body exposures. Furthermore, a biological indicator of radiation damage takes individual radiosensitivity into account. Systematic biomonitoring by cytogenetic biological dosimetry is an important tool, complimentary to physical dosimetry, for the follow-up of radiation workers.

The first goal of the project was to optimise, standardise, and validate the cytogenetic techniques mentioned earlier as sensitive and reliable methodologies for assessing radiation induced genetic damage. The second goal was to apply the optimised techniques to biomonitoring radiation workers in the nuclear industry and medical sector.

Materials and methods

In the present research project the following cytogenetic techniques were applied: the micronucleus-centromere technique, chromosomal aberration analysis for dicentrics, and "multicolour" fluorescence in situ hybridisation (FISH) for scoring translocations. These techniques are applied to peripheral blood lymphocytes. For the micronucleus-centromere technique a blood culture is initiated with phytohaemagglutinin used as mitogen. Cytochalasin B is added 42 hours after the start of the cultures to block cytokinesis. After 70 hours of incubation, cells are harvested, treated with a hypotonic solution, and fixed with methanol-glacial acetic acid. In situ hybridisation is performed using an all-chromosomes centromeric DNA probe. For this hybridisation the research group in Gent used the p82H probe, while

the team in Brussels used the SO αAllCen probe. Immunofluorescence detection of the probe was performed by means of FITC with propidium iodide as counterstain. For scoring micronuclei in binuclear cells and detecting centromeres the preparations were examined under a fluorescence microscope.

For the chromosome aberration analysis, performed by the research group at the University of Liège, blood cultures were incubated for 52 hours including a 75-min colcemid treatment before harvest. The cells were then treated with a hypotonic solution and fixed with methanol-acetic acid. Conventional staining for the standard analysis of dicentrics is based on the fluorescence-plus-Giemsa technique. For FISH analysis of chromosome aberrations a cocktail of probes specific to chromosomes 2, 4, and 8 was used. For scoring translocations and other chromosome aberrations, only cells complete with 46 centromeres were counted.

The micronucleus-centromere technique was used to screen a population of radiation workers from the University Hospital Gent and the Doel nuclear power plant. The first study comprised 120 individuals, working in fourteen different departments of the hospital. The group under study consisted of 71 radiation workers, 36 males and 35 females, with a significant radiation burden, while the control population consisted of 49 workers, 12 males and 37 females, not at all occupationally exposed to radiation. Half of the volunteers were non-smokers. The workers had to fill in a form with the information necessary for the study (sex, age, smoking habits, department, medical exposures). The blood samples were taken by the occupational medicine department on the occasion of the periodical medical examination. This department provided, for entry on the form and on the basis of personal dosimetry records, the information regarding the radiation burden cumulated over the last year and the last ten years.

For the study of the 215 workers of the Doel nuclear power plant, a similar procedure was followed. After written informed consent, the workers filled in a questionnaire, as in the case of the study of the medical workers, and a blood sample was taken on the occasion of the periodical medical examination. All participants were males. Two procedures were followed for the analysis of the radiation burden data. The first was based on the dose cumulated over the last ten years. In the second, the lymphocyte turnover in the blood was taken into account: the radiation burden cumulated over the last ten years was corrected for the half-life of peripheral blood lymphocytes, 3 years.

In addition to the large studies using the micronucleus-centromere technique, the incidence of translocations was investigated in a smaller group of radiation workers at the nuclear power plant in Tihange. This study comprised 46 workers: a study group of 28 radiation workers with the highest radiation burden according to their records and a control population of 18 workers with a negligible cumulated dose. After written informed consent, the workers filled in a questionnaire as in the other studies, and a blood sample was taken on the occasion of the periodical medical examination. All participants were males. The occupational medicine department provided for entry on the form, on the basis of personal dosimetry records, the information regarding the radiation burden cumulated over the last year, the last three years, and over the entire career.

Informed written consent was given by all participants. The inquiry forms were anonymous. A code was given to each participant by the occupational medicine department and this code was marked on the blood sample for the analysis.

Results

In a collaborative study we have analysed the applicability of the optimised cytogenetic techniques to biomonitoring radiation workers. The analysis was based on in vitro irradiated blood samples from donors. Apart from reproducibility of dose-dependence, the most important features required of a biomonitoring technique are high sensitivity and rapid and easy applicability. Given the sample size (a few hundred workers per year) the scoring time for each individual has to be restricted to one day.

The relatively easy and rapid scoring of micronuclei makes this method very attractive for biomonitoring large populations. The present study shows that the dose limit for unequivocal detection at the 95 % confidence level in an individual case is 2 Sv when scoring 2000 binuclear cells. With the micronucleus-centromere technique, the detection limit is lowered to 0.1 Sv. The additional study complementary to the project has shown that a further increase in sensitivity can be expected from selecting the B-lymphocytes. Conventional analysis of dicentrics allows scoring of 200-300 metaphases on two slides per day. Taking into account the inter-individual variance seen in this study with restriction of the scoring time, the detection dose limit for conventional analysis for dicentrics is 0.5 Sv. Chromosome painting with different chromosome-specific DNA probes provides a new technique for the rapid and accurate

detection of translocations. For translocation analysis of the blood samples in the present study, chromosomes 2, 4, and 8 were painted, representing 19 % of the genome. Scoring of two slides takes about one day. This corresponds to about 400 cells for control samples and 100 cells for irradiated samples. Compared to conventional scoring of dicentrics, scoring of translocations by painting chromosomes 2, 4, and 8 has a higher detection limit: 1 Sv. The fact that only 30 % of all translocations can be scored with this painting system is responsible for the difference: the problem of the statistical uncertainty for biodosimetry is harder with painting than with conventional analysis of dicentrics. Furthermore, inter-individual differences in the spontaneous rate are greater for translocations than for dicentrics. It is clear that for practical reasons low-dose-range chromosome painting is currently restricted to a number of selected individuals and to radiation accidents. The fact that translocations act as a cumulative dosimeter because of their temporal stability remains a very important characteristic of this biological indicator of genetic radiation effects.

In a second stage of the research project, the optimised cytogenetic techniques were used to screen several populations of radiation workers. A large population of workers in the medical sector (120 individuals) and nuclear industry (215 individuals) were studied by means of the micronucleus-centromere technique. The control groups in these studies provided very valuable data on the spontaneous incidence of centromere-positive and –negative micronuclei. Both studies showed that the systematic increase in micronucleus frequency with age, of 0.24-0.31 micronuclei per year, is mainly due to increased chromosome loss, reflected by the centromere positivity of the micronuclei. Micronucleus frequencies were 30 % higher in females than in males, a fact again attributable to higher chromosome loss, very probably of the X-chromosome. The data bank resulting from these studies will be of great value for screening populations exposed to radiation. A restricted number of individuals showed an exceptionally high micronucleus frequency (more than 50), almost completely centromere-positive. These individuals belonged to the control group as well as to the population under study.

The analysis of the data on the influence of smoking habits on the micronucleus frequency gave negative results: no correlation between smoking and an enhanced number of centromere-positive or –negative micronuclei was observed. Likewise, after classification of the workers according to their radiation burden, no statistically significant differences showed up within 95 % confidence limits. For the population of medical radiation workers a slightly enhanced number of centromere-positive micronuclei was observed. On the other hand, a linear regression applied to the data of the radiation workers in the nuclear industry showed a slight increase in the number of centromere-negative micronuclei with the dose (0.10 micronuclei per mSv). This value is in very good agreement with the dose dependence found in a previous study of radiotherapy patients (0.13 micronuclei per mSv) and supports the hypothesis of the non-existence of a dose threshold for the clastogenic action of radiation. Apart from exposure to chemicals with aneugenic action, genomic instability is a possible explanation for the enhanced incidence of centromere-positive micronuclei in the medical radiation worker population.

With the elaborated painting technique for chromosomes 2,4 and 8, a comparative study was carried out on a smaller scale, the aim being to compare numbers of translocations in a control population and a group of radiation workers in the nuclear industry with the highest radiation burden. This study showed an increased genomic translocation frequency in the latter group, although statistical significance was not reached. The fundamental problem is that translocation scoring is time-consuming.

Conclusions

The present research project has enabled the acquisition of methodologies and know-how for biomonitoring radiation workers, also applicable to radiation accidents. Currently only the micronucleus-centromere technique can be used to biomonitor relatively large populations. For radiation workers with high exposure this technique could replace the blood count, currently applied as a radiation-specific investigation in the periodical medical examination. In the context of prevention, this should improve the quality of the individual medical follow-up of radiation workers. In view of implementing cytogenetic biomonitoring at the workplace, the inherent ethical-social aspects must also be investigated.