Stable chromosome aberrations 25 years after severe accidental radiation exposure

A. Maes¹, A. Hilali², E.D. Léonard², A. Léonard², L. Verschaeve¹

¹ Vlaamse Instelling voor Technologisch Onderzoek (V.I.T.O.), Division of Environmental Research, Boeretang 200, B-2400 Mol
² Université Catholique de Louvain, Teratogenesis and Mutagenesis Unit, B-1200 Brussels, Belgium

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Abstract. A thorough cytogenetic analysis using G-banding was performed on 100 peripheral blood lymphocytes from an individual who had been accidentally exposed to radiation more than 25 years previously. More than 60% of the analysed cells were found to possess one or more stable chromosome aberrations (e.g. reciprocal translocations). Chromosomes 1 and 11 were more involved in these aberrations than would be expected from the relative chromosome lengths. No identical stable aberrations were found, suggesting that, 25 years after near-lethal exposure, haemopoietic stem cells display substantial diversity.

Introduction

A person accidentally irradiated more than 25 years ago was studied repeatedly over the years and displayed apparently identical translocations (Léonard et al. 1988). The studies were performed on uniformly stained chromosomal preparations. However, it has been repeatedly shown in the literature (e.g. Ohtaki et al. 1982) that it is almost impossible without the application of a banding procedure to clearly identify chromosomes, especially to determine whether or not two similar aberrations are identical. Therefore confirmation by a careful analysis using banding procedures is necessary.

Such an irradiated subject is ideally suited for investigating whether the stable aberrations involve the same chromosomes and the same breakage and recombination sites. Data from a karyotypic analysis of this person thus provide a unique example in considering the appearance of clones in haemopoietic stem cells. We here report the results of such a banding analysis.

Correspondence to: L. Verschaeve
Materials and methods

A 58-year-old man was accidentally exposed in 1965 at the Nuclear Center in Mol to a mixture of gamma-rays and neutrons yielding an average whole-body absorbed dose of about 5 Gy. However, the irradiation was very inhomogeneous, doses received varying from 2 Gy at the head to 50 Gy at the left foot (Léonard et al. 1988).

Peripheral blood was cultured according to standard methods (IAEA 1986) and metaphases were harvested after 48 and 72 h. Three types of cytogenetic analyses were performed on well-spread and well distinguishable chromosomes: chromosome aberrations, chromosome banding and sister chromatid exchanges (SCE). Chromosome aberrations were studied in Giemsa-stained metaphases, 362 and 200 metaphases were analysed in the 48- and 72-h cultures respectively. A more detailed analysis of stable aberrations and the location of breakpoints was performed on 100 trypsin-banded metaphases using a slightly modified standard method (Seabright 1971): freshly spread slides were artificially aged by placing them for 1 h on a hotplate (120°C). Afterwards, the slides were treated with a trypsin solution, washed in saline and finally stained with a 2% Giemsa solution in Sorensen's buffer (pH = 6.8). Full karyotyping was performed on photographic prints of 100 well-banded, well-spread metaphases from the 48- or 72-h cultures. In parallel, 48- and 72-h cultures were also set up in the presence of bromodeoxyuridine for the analysis of the cell kinetics and the frequency of SCE. These slides were stained with a standard fluorescence plus giemsa (FPG) method as modified from Perry and Wolff (1984). The cells were treated with a Hoechst solution in the dark, and then put under white light for 24 h before treating with a sodium saline citrate (SSC) solution at 65°C and stained with a 5% Giemsa solution in SSC. The cell kinetics were studied on 200 metaphase figures after 48 and 72 h culture time; the SCE frequency was evaluated on 50 second-division metaphases (M2).

Results and Discussion

The present data extend our previous investigations on chromosome aberrations in a person exposed accidentally to ionizing radiation more than 25 years ago (Léonard et al. 1988). This person, who has died in the meantime, was still under medical supervision and treatment and was taking two types of insulin for the treatment of a diabetic condition and another product to stimulate liver function. However, none of the compounds could theoretically be considered to be clastogenic or to have “radiomimetic” properties. This was confirmed by the analysis of unstable chromosome aberrations and SCE, which are very sensitive to most chemical mutagens but react little to ionizing radiations. Only 0.6 and 3% gaps, and 0.6 and 0% dicentrics were found in the 48- and 72 h cultures respectively, whereas the frequency of SCE was found to be 5.54±0.3 (standard error) per cell which corresponds to the control levels observed in our laboratory (e.g. Deknudt 1986). The same holds true for the cell kinetics as expressed by percentages of first, second and subsequent cell divisions, found to be 96, 4, and 0% in 48-h cultures and 25, 37 and 38% in 72-h cultures.

In this study we used a G-banding procedure to reveal the presence and type of stable chromosome aberrations resulting from the radiation exposure.