Mutation Research 755 (2013) 68–72

Contents lists available at SciVerse ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Cytogenetic analysis in 16-year follow-up study of a mother and fetus exposed in a radiation accident in Xinzhou, China

Qing-Jie Liu[a,*], Xue Lu[a], Hua Zhao[a], Sen Chen[b], Ming-Ming Wang[c], Yushu Bai[a], Shu-Lan Zhang[b], Jiang-Bin Feng[a], Zhao-hui Zhang[b], De-Qing Chen[a], Li-Wen Ma[b], Ting-Zhen Jia[b], Li Liang[b,**]

[a] China CDC Key Laboratory of Radiological Protection and Nuclear Emergency, National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention, Beijing 100088, PR China
[b] Department of Tumor Chemotherapy and Radiation Sickness, Peking University Third Hospital, Beijing 100191, PR China
[c] Institute of Radiation Medicine, Anhui Medical University, Hefei, Anhui Province 230032, PR China

ARTICLE INFO

Article history:
Received 19 March 2013
Received in revised form 24 May 2013
Accepted 27 May 2013
Available online 3 June 2013

Keywords:
Radiation accident
Prenatal exposure
Cytogenetic analysis
Fluorescence *in situ* hybridization
Dose estimation

ABSTRACT

In November 1992, a radiation accident occurred in Xinzhou, due to the collection by a farmer of an unused ^{60}Co source; 37 individuals were exposed to ionizing radiation. Three individuals died and the farmer's 19-weeks-pregnant wife suffered acute radiation symptoms. Conventional chromosome analysis, cytokinesis-block micronuclei (CBMN) assay and fluorescence *in situ* hybridization (FISH) painting with three pairs of whole chromosome probes were used to analyze chromosomal aberrations for the pregnant female and her baby during the 16 years following the accident. The yields of dicentrics and rings (dic + r) continually declined between 41 days and 16 years after the accident. The frequency of binucleated MN also decreased over time for both mother and daughter. Sixteen years after exposure, the yields of dic + r and binucleated MN decreased to normal levels, but the reciprocal translocation frequencies remained elevated, for both mother and daughter. FISH results showed a decreasing yield of translocations with time. Based on the changes in maternal translocation frequency, the daughter's dose at the time of exposure was estimated as 1.82 (1.35–2.54) Gy. This was consistent with the clinical manifestations of severe mental retardation and low IQ score. FISH-based translocation analysis can be used for follow-up studies on accidental exposure and, after correction, for retrospective dose estimation for individuals prenatally exposed to radiation.

1. Introduction

Biological dosimetry using the analysis of dicentrics or micronuclei (MN) in human lymphocytes is a well-established method, especially for acute radiation exposure [1–3]. However, dicentrics or MN decline over time and are not appropriate for dose estimation of exposures that occurred more than one month previously. Fluorescence *in situ* hybridization (FISH)-based translocation analysis is a useful method for retrospective dose estimation [4]. Recent evidence, however, shows that the persistence of translocation should also be questioned [5,6].

On November 19–27, 1992, a radiation accident occurred in Xinzhou, Shanxi Province, China [7]. In the morning, a farmer, unaware of the hazard, picked up an unused 396 GBq Cobalt-60 source and kept it in his jacket pocket. He was sent to hospital, after complaining of nausea and vomiting later in the afternoon. The jacket with the source remained with him during his hospitalization and altogether thirty-seven individuals were exposed to radiation during this period [8]. He died on December 2, 1992. His father and elder brother, who had been taking care of him at the hospital, both died during the following week.

His wife ("M") assisted with the care of her husband and was intermittently exposed to the source for several days when she was 19 weeks pregnant. She requested medical assistance in Beijing on December 17, 1992. The doctors suspected that she was suffering from radiation exposure. A peripheral blood sample was collected from "M" for biological dose estimation on December 30, the 41st day after the accident. Her whole-body biological dose was estimated at 2.30 Gy (95% CI: 2.07–2.50 Gy) using lymphocyte chromosome analysis [9]. The equivalent whole-body dose weighted by hematological stem cell survival was estimated to 2.0 Gy and the estimated dose of uterus was 1.4 Gy. Considering the clinical symptoms (fever, hair loss, and bone marrow hypoplasia) and physical and biological dose estimations, "M" was diagnosed

* Corresponding author at: National Institute for Radiological Protection, China CDC, 2 Xinkang Street, Deshengmenwai, Beijing 100088, PR China. Tel.: +86 10 62389629; fax: +86 10 62012501.
** Corresponding author.
E-mail addresses: qjliu@nirp.cn, qingjieliu@yahoo.com (Q.-J. Liu), liang_bjmu@hotmail.com (L. Liang).

1383-5718/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.mrgentox.2013.05.015

with the moderate bone marrow form of acute radiation sickness. She recovered after comprehensive treatment with antibiotics, blood transfusion, and hematopoietic growth factor infusion [10].

An ultrasonography examination at 36-week gestational age showed intrauterine growth retardation of the fetus. A baby girl ("B") was born on March 24, 1993 at 37 weeks gestation by artificial rupture of the fetal membranes and labor. The Apgar score for the infant was 10 at both 1 min and 5 min after birth. "B" had little hair, which easily fell out upon touch [11].

The present follow-up studies were performed to monitor the cytogenetic changes in "M" since the 41st day after the accident. The follow-up analysis for "B" had been focused on her physical development during the first seven years after the accident and cytogenetic changes, which have also been analyzed since she was 7.2 years old. The cytogenetic analysis techniques used in the present study included the following: conventional chromosome aberration analysis (dicentric plus centric ring, dic + r); the cytokinesis-block micronucleus (CBMN) assay; and translocation frequency by single color fluorescence *in situ* hybridization (FISH) painting with three pairs of whole chromosome probes. The dose absorbed at the time of exposure for "B" was also estimated in the present study, when the daughter was 16 years old, using FISH analysis.

2. Materials and methods

2.1. Subjects and clinical data

Clinical follow-up studies on "M" and "B", 16 years after the accident, were previously described [12,13]. Briefly, "M" often felt weak and had frequent colds after she recovered from acute radiation sickness. Her hair turned gray, without apparent hair loss, when she was 32 years old, 9 years after the radiation accident. Her menstrual period was reduced from 5–6 days to 3 days during the 16 years after the accident. Sixteen years after the accident, she was diagnosed with bilateral thyroid enlargement (degree I). Laboratory studies showed that the levels of triiodothyronine, total thyroxine, free triiodothyronine and free thyroxine were increased while thyrotropin decreased. Antithyroid peroxidase antibody levels were also elevated. These symptoms indicated that she had hyperthyroidism and chronic thyroiditis. No apparent abnormalities were found in the major organs. No malignant tumor was detected.

The follow-up study on "B" was focused on physical development during the first 7 years. A small head circumference was found at each follow-up except 4 months (Table 1). She appeared healthy, normal, although she often caught cold until she was 4 years and 7 months old. Her grades in school were poor, particularly in mathematics. She was unable to perform simple addition and subtraction. At 16 years old, when she converses with other people, she can only answer simple questions. The results of the China-revised Wechsler Intelligence Scale for Children test (C_WISC) for general cognitive ability showed that verbal, performance, and full-scale IQ scores were 51, 50, and 46, respectively, by which we would classify her as having moderate mental retardation. There is no evidence of other developmental abnormality at age 16.

2.2. Blood sample collection

This work was conducted at the National Institute for Radiological Protection (NIRP), Chinese Center for Disease Control and Prevention. The Ethics Committee of NIRP approved all analyses in the present study. The scope of the study was explained to each subject and written informed consents were obtained. Approximately 2–5 ml peripheral blood was collected at each follow-up, with heparinized syringes or heparinized vacutainer tubes (Becton-Dickinson, USA) by venipuncture.

2.3. Chromosome preparation, unstable chromosome aberration analysis and dose estimation

Unstable chromosome aberration analyses were performed for "M" at 41, 53, 73, and 117 days, 7.5 years, and 16 years after the accident. For "B", unstable chromosome aberrations were analyzed at 7.5 and 16 years after the accident.

Approximately 1 ml peripheral blood was divided into two parts and cultured in 5 ml RPMI-1640 culture medium containing 0.2% phytohemagglutinin (PHA, Invitrogen, Carlsbad, USA), antibiotics and 20% fetal calf serum (HyClone, USA). Colchicine (Shanghai Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) was added immediately to a final concentration of 0.04 μg ml^{-1}. The mixtures were incubated at 37 °C for 52 h. The cells were then collected and treated with a hypotonic solution (0.075 M KCl) for 30 min at 37 °C, followed by two rounds of fixation with methanol–acetic acid (3:1, v/v). Slides were prepared in ice-cold humid conditions and stained with Giemsa. Dicentrics and rings (dic + r) were counted under a light microscope (1000×, Olympus BX51, Japan).

A dose–effect curve obtained from *in vitro* irradiation experiments, ^{60}Co γ-rays, 0.5–5 Gy [15] was used to estimate the exposure dose of the victims. The dose–effect relationship was $Y = 0.035\ D + 0.069\ D^2$ ($R^2 = 0.998$), where Y is the number of dic + r per cell and D is the dose in Gy. Another dose-effect curve, for dose range 0.1–0.5 Gy [15] ^{60}Co γ-rays, was used to estimate the absorbed dose for the victim if, according to the above equation, the estimated dose was lower than 0.5 Gy. This dose–effect relationship was $Y = 0.058 + 0.014\ D + 0.00038\ D^2$ ($R^2 = 0.996$), where Y is the number of dic + r per 100 cells, and D is the dose in cGy.

2.4. Micronucleus analysis and dose estimation

CBMN analyses were performed for "M" at the 41st day, 7.5 years and 16 years after the accident. For "B", only the 7.5 and 16 years post-accident analyses were conducted.

Approximately 1 ml peripheral blood from each victim was added to 4 ml culture medium containing PHA. After 44 h culture at 37 °C, cytochalasin-B (Sigma, St. Louis, MO, USA) was added to a final concentration 6 μg ml^{-1}, and the mixtures were then cultured continuously for another 28 h. The cultured cells were collected and treated with a hypotonic solution (0.075 M KCl) for 2 min at room temperature, then fixed with methanol–acetic acid (3:1). Slides were prepared as described above and stained with Giemsa. Binucleated cells were scored under a light microscope. The numbers of micronuclei in binucleated cells were recorded.

A micronucleus dose–effect curve was used to estimate the exposure doses of the victims. The dose–effect relationship was as follows: $Y = 17.91 + 3.38\ D + 42.88\ D^2$ ($R^2 = 0.997$), where Y is the number of micronuclei per 1000 cells, and D is the dose in Gy; the dose range could be estimated from 0.1 to 5 Gy [16]. Another dose-effect curve for the dose range from 0.1 to 0.5 Gy [16] was used to estimate the absorbed dose for the victim if the estimated dose, according to the above equation, was lower than 0.5 Gy. The dose–effect relationship was $Y = 14.99 + 38.43\ D$ ($R^2 = 0.995$), where Y is the number of micronuclei per 1000 cells, and D is the dose in Gy.

2.5. Fluorescence in situ hybridization, translocation analysis and dose estimation

FISH-based translocation analysis was carried out at 7.5 years and 16 years after the accident for "M". Analysis was performed for "B" only at 16 years after the accident.

Approximately 3 ml peripheral blood from "M" and "B" was used to isolate the lymphocytes with Ficoll-Paque (Amersham Pharmacia Biotech). Cells were washed three times with fresh RPMI 1640 medium (Invitrogen, Carlsbad, USA), and chromosome preparations were prepared by adding colchicine at the start of culturing, according to the method described in Section 2.3. A few drops of cell suspension in fixative were dropped onto dried, alcohol-pre-cleaned slides. The slides were then placed above a container filled with heated water for 20–60 s, depending on the ambient humidity [17]. The remaining cell suspension was kept at −20 °C.

Each slide for FISH was pretreated with RNase A (100 μg ml^{-1} in 2× SSC, Boehringer Mannheim, USA) and proteinase K (Sigma–Aldrich, Santa Clara, USA) and fixed in 1% formaldehyde according to the method of Pinkel et al. [18]. The slides were denatured with 70% formamide and 2× SSC at 70 °C for 1–2 min, followed by dehydration by an ice-cold ethanol series (70%, 90% and 100%). The denatured probes, which were directly labeled with FITC (Cell Bank, Kuming Institute of Zoology, Chinese Academy of Sciences), of chromosomes 1, 2 and 4, were applied to these pretreated chromosome slides and hybridized at 37 °C overnight. Three post-hybridization washes were performed at 42 °C in 50% formamide and 2× SSC for 5 min each, followed by three washes in 0.1× SSC at 50 °C for 5 min each. Chromosome counterstaining was performed with 0.15 μg ml^{-1} 4′,6-diamidino-2-phenylindole (DAPI). The slides were stored in a dark −20 °C freezer before analysis.

The FISH slides were viewed with a Zeiss Axioplan 2 Imaging fluorescence microscope (Zeiss, Oberkochen, Germany) with a cooled charge-coupled device (AxioCam HRM, Zeiss) and were analyzed using Isis FISH analysis software (Metasystems, Germany). Two-color (green and blue) images were captured, merged and stored. Two thousand cells were scored for each sample.

Metaphases were considered suitable for analysis only if they appeared to be complete, the fluorochrome signals were sufficiently bright, the centromeres were morphologically detectable and all three pairs of chromosomes appeared somewhere in the cell. Generally, the scoring criteria followed the conventional scoring system. Each translocation was checked by two observers. The cells were scored as normal when all signals on the three pairs of chromosomes were complete. A reciprocal translocation was recorded if more than six chromosomes were observed with green color and two chromosomes were observed with green-blue color.

The FISH results were converted into the genome translocation frequency using the Lucas formula $F_G = F_p/2.05f_p(1 - f_p)$ [4], where F_p is the translocation frequency detected by FISH, F_G is the full genome translocation frequency, and f_p is the fraction of the genome hybridized, which is 22.3% for chromosomes 1, 2 and 4 of female [19].

A dose–effect curve obtained during FISH-based translocation analysis was used to estimate the exposure doses of the victims. The dose–effect relationship was as follows: $Y = 0.0037 + 0.0062\ D + 0.043\ D^2$ ($R^2 = 1.000$), where D is the absorbed dose in Gy for the genome translocation frequency Y. The dose range could be estimated from 0.5 to 5 Gy [20].