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Unusual response to bifunctional alkylating agents in a case of Fanconi anaemia

M. L. Kwee[1], E. H. A. Poll[1], J. J. P. van de Kamp[2], H. de Koning[3], A. W. Eriksson[1], and H. Joenje[1]

[1] Institute of Human Genetics, Free University, P.O. Box 7161, NL-1007 MC Amsterdam, The Netherlands
[2] Clinical Genetics Centre, Academic Hospital, Rijnsburgerweg 10, NL-2333 AA Leiden, The Netherlands
[3] Department of Pediatrics, Academic Hospital, Rijnsburgerweg 10, NL-2333 AA Leiden, The Netherlands

Summary. Chromosomal breakage frequencies were determined in Fanconi anaemia (FA) blood cultures treated with various concentrations of the polyfunctional alkylating agents mitomycin C, diepoxybutane, and *cis*-platinum(II)-diammine-dichloride, for which FA cells have a characteristic hypersensitivity. At concentrations that hardly affected control cultures, three out of four patients tested exhibited a concentration-dependent increase of cells with aberrant chromosomes, with a concomitant increase in the number of chromosomal aberrations per aberrant cell. The fourth patient, a 22-year-old male, was exceptional because with all three clastogens only 40% of his cultured cells exhibited a typical concentration-dependent response, while 60% of his cells responded like those from normal healthy controls. The possible nature and significance of this unusual response is discussed.

Introduction

Fanconi anaemia (FA), is an autosomal recessive disease characterized by low birth weight, growth retardation, aplastic anaemia (often apparent in the first decade of life), variable congenital malformations (especially of the radius, thumb, kidney), skin pigment disorders (Fanconi 1927, 1967; Glanz and Frazer 1982), and spontaneous chromosomal instability (Schroeder et al. 1964). Marrow failure may lead to death within a few months or years. In affected individuals there is an increased incidence of malignancy (acute leukaemias, hepatomas, squamous cell carcinomas) (Swift 1971; Schroeder and Kurth 1971; German 1972; Schroeder 1982).

The variability in expression of the disease often makes clinical diagnosis difficult. Increased spontaneous chromosomal breakage generally found in cultured peripheral blood lymphocytes has been of great value in confirming the diagnosis (Schroeder et al. 1964; Bloom et al. 1966; Swift and Hirschhorn 1966), but this feature also shows considerable variation. Possibly the most consistent feature of the disease is a hypersensitivity of FA cells to the clastogenic action of bifunctionally alkylating (cross-linking) agents, such as mitomycin C (MMC) (Sasaki and Tonomura 1973), diepoxybutane (DEB) (Auerbach and Wolman 1976) and *cis*-platinum(II)-diammine-dichloride (cisPtII) (Poll et al. 1982).

Hypersensitivity towards cross-linking agents has been proposed as the best criterion to distinguish FA from related disorders (Auerbach et al. 1981; Cervenka et al. 1981). Here we present a case of FA who showed hypersensitivity to bifunctional alkylating agents in only a minority of his cultured lymphocytes, the majority being as sensitive as those from healthy control individuals.

Offprint requests to: M. L. Kwee

Materials and methods

Peripheral blood samples were obtained from four FA patients, five healthy control individuals and two obligate heterozygotes (parents of FA patient case 2).

Case 1. JdW (II-4 of Fig. 1), born in 1960, was the third child of healthy unrelated parents. The first child of the family (II-1) had a bilateral thumb aplasia and esophageal atresia and died 2 days after birth. Chromosomal studies were not done. The second pregnancy (II-2) ended in an abortion. The second child (II-3) is healthy. The fourth child (II-5), born in 1962, had a birth weight of 2350 g. At the age of 5 years she developed granulocytopenia and later pancytopenia. She had bilateral thumb aplasia, café-au-lait spots, ventricle septum defect, short stature and an increased chromosomal breakage rate. On these criteria a diagnosis of FA was made. She died at the age of $11\frac{1}{2}$ years with incurable aplastic anaemia.

The proband (II-4) was first examined and diagnosed at the age of $7\frac{1}{2}$ years, after FA had been diagnosed in his sister (II-5). His birth weight was less than 2500 g and length 48 cm. He had thumb aplasia and radius hypoplasia on the left side and a short stature. Intravenous pyelography revealed a normal right kidney and a normal but rather small left kidney. His haematological data consistently showed slight anaemia and granulocytopenia. During 14 years follow-up, there have been periods of slight to severe thrombocytopenia; blood transfusion has never been necessary.

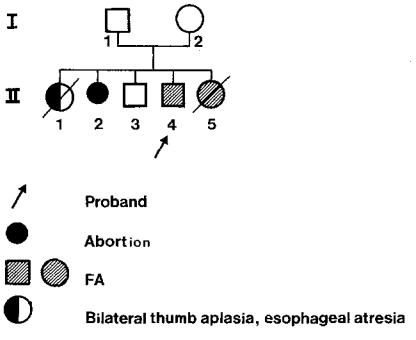

Fig. 1. Family data of case FA-1

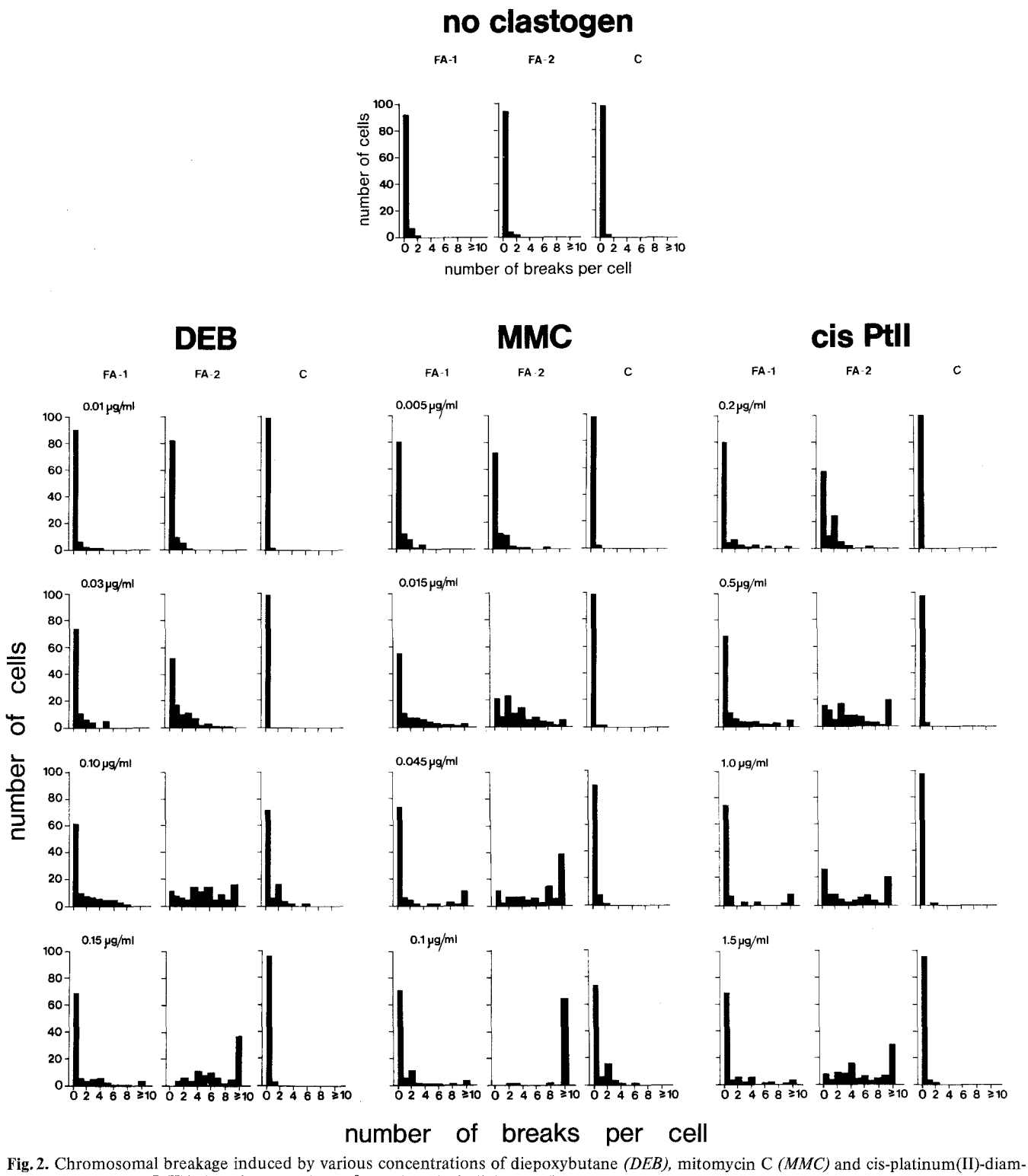

Fig. 2. Chromosomal breakage induced by various concentrations of diepoxybutane *(DEB)*, mitomycin C *(MMC)* and cis-platinum(II)-diammine-dichloride *(cisPtII)* in lymphocyte cultures from a healthy individual *(C)*, a typical FA case *(FA-2)* and the unusually responding case *(FA-1)*, as determined in a single experiment

Case 2. JV, a girl born in 1979 after 36 weeks of pregnancy; her birth weight was 2180 g. She had several congenital malformations (ventricle septum defect, peripheral pulmonary stenosis, aplasia of vagina and uterus, unilateral radial and thumb aplasia, unilateral short ulna, aplasia of the right kidney, rib anomaly), growth retardation and chromosomal instability. The diagnosis was made at 1 month of age, in the preanaemic phase. Her parents are healthy and unrelated; she has two healthy sisters.

Case 3. MM, a girl born in 1971. She had a persistent ductus Botalli which was surgically corrected. Other congenital malformations were absent. She had symptoms of thrombopenia and leucopenia.

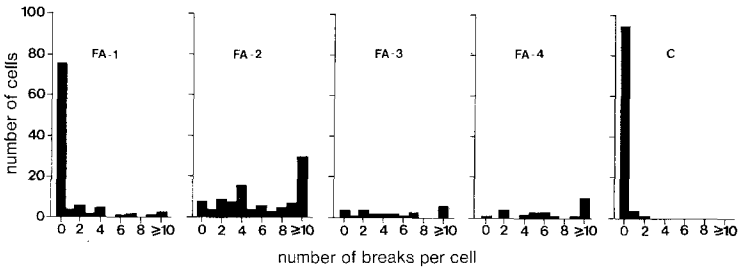

Fig. 3. CisPtII-induced chromosomal breakage in lymphocyte cultures from four FA patients and a healthy control. CisPtII concentration was 1 μg/ml for FA-3 and FA-4, and 1.5 μg/ml for the other individuals. Note the unusual response in case FA-1

Case 4. RM, a brother of MM (case 3), born in 1968 as one of dizygotic twins. His twin brother died suddenly of an unknown cause at the age of 9 months. RM developed acute myeloid leukaemia at the age of 12 years. After treatment he is now still in remission.

In all four FA patients the diagnosis had been confirmed by an increased frequency of spontaneous and/or MMC-induced chromosomal aberrations.

Methods. Fresh heparinized whole blood (0.5 ml) was added to 4.5 ml Ham F10 medium containing 15% fetal calf serum, antibiotics, and phytohaemagglutinin (PHA, Wellcome Laboratories), incubated at 37° C for 72 h, harvested and processed for chromosomal analysis following standard methods (Moorhead et al. 1960). Where possible, 100 Giemsa-stained metaphases were scored for chromosomal aberrations, on coded slides.

For the quantification of chromosomal damage, metaphases with less than 40 chromosomes, tetraploids and endoreduplications were excluded from the analysis. Aberrations were converted to breaks as follows. Chromatid and isochromatid breaks were considered as a single break event; dicentrics, triradials and quadriradials as two break events; more complex interchanges as the lowest possible number of breaks required for their reconstruction. Gaps, identified according to the International System for Human Cytogenetics Nomenclature (1978), were excluded from the analysis.

Clastogen treatment. Blood cultures were treated with various concentrations diepoxybutane (DEB, purchased from Merck), mitomycin C (MMC, from Kyowa Hakko Kyogo Co. Ltd., Tokyo) and cisPtII (Bristol-Meyers). MMC and cisPtII were added at the time of culture initiation; DEB was added 24 h after initiation.

Results

In lymphocyte cultures from each of the four FA patients we observed increased spontaneous and drug-induced chromosomal breakage rate when compared to normal controls. While in the higher concentration range virtually all cells from three of the FA patients could be induced to contain one or more aberrations, in FA case 1 there was still a large proportion (ca. 60%) of cells that failed to show any damage, even at the highest concentration of clastogen. This phenomenon is illustrated in Fig. 2, which shows concentration-dependent chromosomal breakage in a typical FA patient (case 2), the unusual case 1, and a control individual, as determined in a single experiment. Fig. 3 summarizes the results so far obtained with the highest concentration of cisPtII in four FA patients, clearly illustrating the unusual response in case 1. These data suggest that in case 1 *two lymphocyte populations are present,* one being as sensitive to bifunctional alkylating agents as those of other FA patients, and another subpopulation being as sensitive as normal controls.

Lymphocyte cultures from two heterozygotes (parents of case FA-2), when exposed to MMC, DEB and cisPtII at the highest concentrations given in Fig. 2, did not show greater sensitivity towards these agents than controls (cf Auerbach et al. 1981). Unfortunately, blood samples from the parents of case FA-1 were not available for analysis.

Discussion

The clinical symptoms, family history and spontaneous chromosomal aberrations in case 1 justify the diagnosis of FA in this patient. Moreover, in an earlier study (Joenje et al. 1979), erythrocyte superoxide dismutase levels were below normal in this patient, as was the case for six other FA patients. Also, "spontaneous" chromosomal breakage in lymphocyte cultures from this patient responded to oxygen tension over the culture, as did cultures from three other FA patients in the same study (Joenje et al. 1981), further substantiating the validity of the FA diagnosis for case 1. Cytogenetic hypersensitivity to cross-linking agents was also clearly present, but our data show that this response was restricted to only a minority of the cultured lymphocytes, a phenomenon that was not seen in three other FA cases tested similarly. We do not know whether the unusual response was a stable characteristic of case 1, as earlier tests were not done on clastogen concentrations allowing a conclusion on this point. However, considering the family history, it seems very likely that this patient is an FA homozygote and that *the clastogen resistant cells in this patient have arisen de novo* at some stage in his life. Although we have no direct evidence for it, we entertain the possibility that in this patient a bone marrow stem cell may have undergone a genetic change resulting in an increased (i.a. normal) clastogen resistance and an increased rate of proliferation. This cell could have given rise to a clone of genetically "corrected" stem cells that may now be slowly replacing the affected bone marrow stem cells. If this hypothesis of "autotransplantation" is correct, a progressive increase of the fraction of clastogen-resistant lymphocytes is to be anticipated. Follow-up studies are required to elucidate this point.

Auerbach et al. (1981) have mentioned a 9-year-old girl with FA whose lymphocyte cultures showed only 20% DEB-responsive cells. This raises the question whether the occurrence of clastogen-non-responsive cells, as documented here, may be more common among FA patients, possibly reinforced by the increased spontaneous genetic instability characteristic to this syndrome. As the usual cytogenetic tests for FA diagnosis are generally not designed to detect these unusual cases, similar cases could have escaped detection. In addition, the presence of nonresponsive cells in a patient could present a serious risk for misdiagnosis: metaphases from clastogen-hypersensi-

tive FA cells may be under-represented or even absent as a result of an increased mitotic delay compared to cells with normal clastogen sensitivity (Miura et al. 1983).

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