# CYTOGENETIC SENSITIVITY OF THREE FANCONI'S ANEMIA HETEROZYGOTES TO BLEOMYCIN AND IONISING RADIATION

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**Abstract**

It has been widely described that Fanconi's anaemia patients show a hypersensitivity to the effect of cross-linking agents like mitomycin C and diepoxybutane, while the sensitivity of these patients to ionising radiation is controversial. Fanconi's anaemia heterozygotes do not show a hypersensitivity to the agents above mentioned.

On the other hand, bleomycin it is used to identify mutagen sensitive individuals, specially among head and neck cancer patients.

We present here a preliminary study in which the mean frequencies of G2 bleomycin induced chromatid breaks (ctb) from three FA heterozygotes ( $\bar{X}$=0.9, range 0.80-1.01) and eleven controls ( $\bar{X}$=0.40, range 0.21-0.66) differ significantly. Moreover, no overlap was observed between the 95% confidence intervals of both populations, indicating a hypersensitivity of G2 lymphocytes from these three FA heterozygotes to bleomycin (BLM). These hypersensitivity was not observed after a exposure of G0 lymphocytes to 2 Gy of ionising radiation.

## Introduction

Fanconi's anaemia (FA) is an autosomal recessive genetic disease characterised by predisposition to bone marrow failure and leukemia development. FA patients also show high levels of spontaneous chromosome breakage [1] and an increased chromosomal sensitivity to DNA cross-linking agents like mitomycin C (MMC) and diepoxybutane (DEB) [2,3]. Their sensitivity to ionising radiation is controversial [4-7].

Heterozygotes for this disease do not show an increased chromosomal sensitivity to the above mentioned agents, and cannot be differentiated from the normal population.

On the other hand Bleomycin (BLM) is has been used to identify susceptibility to cancer of upper aerodigestive tract [8] and lung carcinoma [9]. BLM has also been used to detect mutagen susceptibility of head and neck cancer patients (??????), and a possible genetic cancer susceptibility has also been described [10-12].

The aim of the present study is to test if the exposures to BLM of G2 lymphocytes or to 2 Gy of $\gamma$-rays of G0 lymphocytes allows to discriminate FA heterozygotes from a control population.

## Materials and methods

*Sampling and culture conditions*

Blood samples from three FA heterozygotes were collected by venipuncture into heparinized tubes. For each individual, three lymphocyte cultures were carried out. The first was used as control, the second was done to know the effect of 2 Gy of γ-rays on G0 peripheral blood lymphocytes, and the third to know the effect of BLM in G2. In all cases, 0.8 ml peripheral blood was cultured for 48 hours in 5 ml RPMI-1640 medium supplemented with 20% of foetal calf serum, antibiotics and phytohemaglutinin. Colcemid was added two hours before harvesting. To select first division metaphases, 12 μg/ml of bromodeoxyuridine were present since the set up of the cultures. Two to three day old slides were stained by the FPG technique [15].

Data on control groups were previously published for both, the 2 Gy [13] and the BLM [14] studies.

*Irradiation and bleomycin treatment*

For the study on the effect of 2 Gy of γ-rays on G0 lymphocytes, peripheral blood samples were irradiated at 2 Gy by a cobalt source (Theratron-780) located at the Hospital de la Santa Creu i Sant Pau (Barcelona). Dose rate ranged from 96.12 to 91.95 cGy/min due to the decay of the source. IAEA recommendations has been followed for the irradiation [16]. For the study on the effect of bleomycin on G2 lymphocytes, a final concentration of 0.03U/ml of BLM was added to the cultures five hours before harvesting.

*Cytogenetic analysis*

Chromosome analyses were carried out exclusively on first division metaphases containing 46 centromeres. All metaphases with chromosome abnormalities were independently analysed by three investigators.

For the 2 Gy irradiated samples, we have attempted to analyse the number of metaphases necessaries to achieve 100 dicentrics. The chromosome-type abnormalities taken into account were: dicentric chromosomes (dic), only considered when the acentric fragment was present, and acentrics plus chromosome breaks, that were recorded together as extra acentrics (ace).

After BLM treatment, we have attempted to analyse the metaphases necessaries to observe about 100 chromatid breaks (ctb), that were scored following the Chatham Barrs Inn Conference [17]

*Statistical analysis*

To check if the distribution of induced dic and ctb among cells followed a Poisson distribution, the dispersion index $D=\sigma^2/y$ and the normalised unit of this index (U) were used [18]. To compare the mean frequencies of FA heterozygotes and controls, the Student's t-test with Satterthwaite's rule was applied.

## Results and discussion

In table 1 it can be seen the cytogenetic results obtained in the three FA heterozygotes after ionising radiation and BLM treatments. As can be seen, the distribution of *ctb* among cells do not follows a Poisson distribution (U values greater than 1.96). On the other hand, and as expected, the distribution of *dic* among cells follows a Poisson distribution.

In table II, it can be seen the comparison between controls and FA heterozygotes. The basal frequencies of chromosomal and chromatid abnormalities were slightly higher in FA heterozygotes than in controls, although the differences were not significant.

After 2 Gy irradiation, the frequencies of *dic* were higher in FA heterozygotes when compared to controls, but the differences were not significant. Moreover an overlap between the 95% confidence intervals from both populations exist. The frequencies of induced extra *ace* were quite similar between both populations. These results are in agreement with other studies that did not find an increased sensitivity to ionising radiation in FA patients [6,7,19,20].

After G2 bleomycin treatment, the frequencies of *ctb*/cell were higher in FA heterozygotes (0.80, 0.89 and 1.01 respectively) than in controls (ranging from 0.214 to 0.664). The mean frequencies of both groups were significantly different and moreover, the 95% confidence intervals from both groups did not overlap.

A study on the immunological phenotype of FA patients and their family members showed that immunological abnormalities may be present in parental heterozygotes of FA patients and that a grading of immunological defects in FA patients and family members exist [21]. This suggestion could agree with the results of the present work, indicating that it could be possible to differentiate FA

heterozygotes, for example by their G2 hypersensitivity to bleomycin. However, the study of more cases is needed.

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**Table 1.-** Cytogenetic results after bleomycin and X-ray irradiation in the Fanconi's anaemia heterozygotes.

| | Cells | dic | ace+csb | r | ctb | gaps | | | | | | | | | | | | | ***y*** | **var** | **U** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | | | | | | | | | | | | | | | | | | | | | |
| MRO | 221 | 2 | 2 | 0 | 3 | 3 | | | | | | | | | | | | | | | |
| PRO | 205 | 0 | 2 | 0 | 1 | 2 | | | | | | | | | | | | | | | |
| MRL | 200 | 0 | 1 | 0 | 2 | 1 | | | | | | | | | | | | | | | |
| **BLM** | | | | | | | Cell distribution of ctb | | | | | | | | | | | | *ctb*/cell | | |
| | | | | | | | *0* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* | *10* | *>10* | | | |
| MRO | 103 | 0 | 1 | 0 | 104 | 29 | 59 | 24 | 8 | 4 | 2 | 3 | 1 | 1 | 0 | 0 | 0 | 1(16) | 1,01 | 4,26 | 23,13 |
| PRO | 210 | 0 | 5 | 0 | 187 | 43 | 134 | 45 | 12 | 4 | 2 | 3 | 1 | 3 | 2 | 2 | 1 | 1(12) | 0,89 | 3,76 | 33,07 |
| MRL | 206 | 0 | 2 | 0 | 164 | 44 | 138 | 33 | 15 | 7 | 3 | 4 | 2 | 2 | 1 | 0 | 0 | 1(14) | 0,80 | 2,94 | 27,39 |
| **2Gy** | | | | | | | Cell distribution of dic | | | | | | | | | | | | | | |
| | | | | | | | *0 dic* | *1 dic* | *2 dic* | *3 dic* | *4 dic* | | | | | | | | *dic*/cell | | |
| MRO | 313 | 104 | 78 | 9 | 8 | 5 | 220 | 82 | 11 | 0 | 0 | | | | | | | | 0,33 | 0,29 | -1,48 |
| PRO | 267 | 100 | 40 | 5 | 8 | 0 | 182 | 72 | 11 | 2 | 0 | | | | | | | | 0,37 | 0,36 | -0,36 |
| MRL | 216 | 68 | 30 | 4 | 8 | 6 | 155 | 54 | 7 | 0 | 0 | | | | | | | | 0,31 | 0,28 | -1,09 |

*dic*=dicentrics; *ace*=extra acentrics; *ctb*=chromatid breaks; var=variance; D=dispersion index; U=Papworth's U

**Table 2.-** Frequencies (x100±SE) of Chromosomal and chromatid abnormalities observed in the control study, after 2 Gy irradiation and after Bleomycin treatment.

| | **Control study** | | | | **Bleomycin treatment** | |
|---|---|---|---|---|---|---|
| | Controls | FAH | Controls | FAH | Controls | FAH |
| Cells analysed | | | | | 2138 | 519 |
| *dic*/cell | | | | | | |
| *ace* /cell | | | | | | |
| *ctb*/cell | | | | | 0.40* | 0.90* |

FAH= Fanconi's anaemia heterozygotes; *dic*=dicentrics; *ace*=extra acentrics; *ctb*=chromatid breaks; *= p<<0.001

**Table 2.-** Frequencies of Chromosomal and chromatid abnormalities observed in the control study, after 2 Gy irradiation and after Bleomycin treatment.

| | | Control group | FA heterozygotes |
|---|---|---|---|
| **Control study** | | | |
| | Cells analysed | 8811 | 626 |
| | *dic*/cell (x100) | 0.09 | 0.32 |
| | *ace*/cell (x100) | 0.57 | 0.80 |
| | *ctb*/cell (x100) | 0.50 | 0.96 |
| **2 Gy irradiation** | | | |
| | Cells analysed | 2504 | 796 |
| | *dic*/cell (95%CI) | 0.31 (0.29-0.33) | 0.34 (0.30-0.38) |
| | *ace*/cell (95%CI) | 0.17 (0.15-0.19) | 0.18 (0.10-0.26) |
| **Bleomycin treatment** | | | |
| | Cells analysed | 2138 | 519 |
| | *ctb*/cell (95%CI) | 0.40* (0.31-0.50) | 0.90* (0.78-1.02) |

FAH= Fanconi's anaemia heterozygotes; *dic*=dicentrics; *ace*=extra acentrics; *ctb*=chromatid breaks; 95%CI= 95% confidence interval; *= p<<0.001

Fig. 1. Individual frequencies of ctb (6 95% confidence interval) are shown by circles and squares in controls and FA heterozygotes, respectively. Mean pop ulation frequencies are shown by black lines, and the 95% confidence intervals by grey lines. Wide and thin lines are for FA heterozygotes and controls, respectively