Effect of Hormones on the Variation of Radiosensitivity in Females as Measured by Induction of Chromosomal Aberrations

C.J. Roberts,1 G.R. Morgan,1 and N. Danford2

1AEA Technology plc, Harwell Laboratory, Didcot, Oxon, United Kingdom; 2Microptic Cytogenetics Ltd, Innovation Centre, Singleton Park, Swansea, United Kingdom

The frequency of dicentrics + ring (dic/cell) and total chromosome aberrations (dicentrics, rings and excessacentrics, etc.) per cell (TAB/cell) has been studied in 50 male and female volunteers after high or low dose rate (HDR, LDR) irradiation of peripheral blood lymphocytes. The mean male aberration frequencies per cell after HDR irradiation were 0.38 dic/cell and 0.61 TAB/cell; following LDR irradiation, the mean aberration frequencies were 0.28 dic/cell and 0.45 TAB/cell. Equivalent female values after HDR irradiation were 0.42 dic/cell and 0.71 TAB/cell; after LDR irradiation, the mean aberration frequencies were 0.30 dic/cell and 0.48 TAB/cell. Analysis of variance showed that there was a highly significant difference between males and females after HDR, but not LDR, irradiation. It is concluded from this study that females have a greater variability in their radioreponse, and that this variability is related to progesterone, which has a profound effect upon radiosensitivity, as measured by cytogenetic end points. — Environ Health Perspect 105(Suppl 6):1467–1471 (1997)

Key words: radiosensitivity, hormones, progesterone, chromosomes, sensitization

Introduction

It has proved difficult to interpret genomic or cellular radiosensitivity in the normal population (1–4). Most work on normal radiosensitivity suggests that interindividual differences are equal to the differences among repeat samples from single individuals (5,6). Consequently, a recent report questioned whether interindividual differences in genomic radiosensitivity are real, suggesting that they may be a consequence of poor experimental reproducibility (7). Several factors contribute to the experimental uncertainty, including nonhomogeneity of the irradiated cells when fibroblasts are used and variation between scorers and experimental conditions in the case of cytogenetic techniques (8).

Recent studies using refined techniques or internal controls for interexperiment variation have suggested that there are real differences in radiosensitivity among individuals (9). Nakamura’s data suggest that lymphocytes may have a more homogeneous radiosensitivity than fibroblasts (3). This observation seemed to be reinforced in a follow-up study of micronucleus formation in peripheral blood lymphocytes by Huber et al. (10), which demonstrated that one individual from a group of four could be identified as significantly more sensitive than the others when replicate experiments were performed. Furthermore, the use of chronic radiation to amplify differences between normal and sensitive groups may prove useful in identifying radiosensitive individuals from the normal population. Paterson et al. (11) used chronic doses of radiation to demonstrate hypersensitivity in cultured fibroblasts derived from AT heterozygotes. Cox (12) also noted that the difference between normal and AT cell lines may be as high as a factor of 15 at low dose rates (about 0.02 cGy/min).

The data described in this paper quantify the effects on levels of chromosome aberrations after acute and chronic irradiation of lymphocytes. This random sample of individuals represents the range of responses that may be found within any general working population. The effect of the female hormone progesterone on radiosensitivity is also reported.

Methods

Initially 6 × 30-ml blood aliquots from 5 healthy male volunteers were sampled randomly over a 4-year period; these volunteers ranged from 30 to 43 years of age. Subsequently, an additional 25 male and 25 female volunteers were selected from normal, clinically healthy individuals ranging from 24 to 63 years of age for the main study. Blood samples were taken into heparinized tubes from each volunteer on two occasions separated by a period of no less than 16 weeks. Careful notes were kept on the medical condition of each donor and on any prescribed medication being taken. Each volunteer completed a questionnaire detailing his/her smoking and alcohol intake, and a note was also made of any occupational exposure to chemical carcinogens and radiation. At each sampling time 2 male and 2 female volunteers were sampled whenever possible.

Hormone Experiments

Progesterone (4-pregnene-3,20-dione; Sigma Ltd, Poole, UK) was diluted with sterile deionized water to give final concentrations of 3, 23, and 46 µg/ml. The hormone was added to whole blood and incubated at 37°C for 2 hr prior to irradiation.

Irradiation Protocol

Irradiations were performed using a 60-cobalt (60Co) γ-ray source. The source has been previously calibrated using standard 100% 7-lithium fluoride (7Li) thermoluminescent dosimeters in the form of a polyethylene container. For the initial experiments on intraexperimental variation, the dose rate for low dose rate (LDR) irradiations was measured at the exposure position as 0.0035 Gy/min, and a total dose of 3 Gy was given over a period of 15 hr. During irradiation, the blood was mixed gently to prevent settling out of the various fractions and was maintained at 37°C. The dose rate of...
for high dose rate (HDR) irradiation was measured as 0.29 Gy/min, and a total dose of 1.75 Gy was administered (6 min). Doses were modified to allow for radioactive decay over the period of these experiments. Unirradiated samples used as controls were also gently mixed and maintained at 37°C.

In the main study the LDR irradiation dose rate measured at the exposure position was 0.0035 Gy/min, and a total dose of 3.99 Gy was administered over a period of 19 hr. During the irradiation the blood again was mixed gently to prevent settling out of the various fractions and maintained at 37°C. The dose rate for HDR irradiation was measured as 0.29 Gy/min, and a total dose of 4.06 Gy was given (14 min). Doses were modified to allow for radioactive decay over the period of these experiments. Unirradiated samples used as a control were also mixed gently and maintained at 37°C.

**Chromosome Assay**

Following stimulation with phytohemagglutinin, cultures were established in growth media RPMI 1640 supplemented with 2.0 g/liter sodium bicarbonate, 20 mM 5-bromo-2-deoxyuridine, 50 IU/ml penicillin, 50 mg/ml streptomycin, and 2 mM L-glutamine. Cultures were maintained at 37°C in a CO2 incubator and sampled between 48 and 52 hr after irradiation. Only cells at the first mitosis were required for cytogenetic analysis and therefore a modified fluorescence plus Giemsa (FPG) staining method (13) was employed. Two hundred first-division cells were selected and analyzed for the presence of chromosome aberrations (dicentrics, rings, double minutes, paired fragments unassociated with dicentrics or rings, clearly distinguishable translocations and inversions, and multicentrics) in control, HDR irradiated, and LDR irradiated cultures. To eliminate any bias during metaphase analysis, all samples were coded and all metaphase analyses were performed by three individuals. For each experimental point each individual scored one-third of the total number of cells required; data were then pooled to give the final total for each sample point.

**Results**

Data from the initial study are listed in Tables 1 and 2. Table 1 shows the levels of aberrations observed in 200 cells analyzed after HDR irradiation of blood samples from five male donors sampled over a 4-year period. Table 2 shows the levels of aberrations observed in 200 cells analyzed after LDR irradiation of blood samples from four.

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**Table 1. Initial study: HDR irradiation data.***

| Identifier | Dic/cell | Error | Tab/cell | Error |
|------------|---------|-------|----------|-------|
| NS12       | 0.27    | 0.04  | 0.45     | 0.05  |
|            | 0.39    | 0.04  | 0.66     | 0.06  |
|            | 0.27    | 0.06  | 0.42     | 0.08  |
|            | 0.24    | 0.06  | 0.42     | 0.08  |
|            | 0.34    | 0.07  | 0.49     | 0.09  |
|            | 0.54    | 0.05  | 0.73     | 0.06  |
| Mean       | 0.34    | 0.04  | 0.53     | 0.06  |
| SD         | 0.11    |       |          |       |
| NS18       | 0.43    | 0.05  | 0.65     | 0.06  |
|            | 0.38    | 0.04  | 0.66     | 0.06  |
|            | 0.48    | 0.05  | 0.67     | 0.06  |
|            | 0.19    | 0.05  | 0.36     | 0.07  |
|            | 0.42    | 0.05  | 0.68     | 0.06  |
|            | 0.36    | 0.04  | 0.53     | 0.06  |
| Mean       | 0.37    | 0.04  | 0.60     | 0.06  |
| SD         | 0.10    |       | 0.13     |       |
| NS15       | 0.47    | 0.06  | 0.67     | 0.07  |
|            | 0.25    | 0.04  | 0.41     | 0.05  |
|            | 0.57    | 0.06  | 0.62     | 0.06  |
|            | 0.43    | 0.05  | 0.59     | 0.05  |
|            | 0.24    | 0.03  | 0.49     | 0.05  |
|            | 0.36    | 0.04  | 0.63     | 0.06  |
| Mean       | 0.36    | 0.04  | 0.57     | 0.06  |
| SD         | 0.09    |       | 0.10     |       |
| NS02       | 0.46    | 0.05  | 0.75     | 0.06  |
|            | 0.36    | 0.05  | 0.65     | 0.06  |
|            | 0.38    | 0.04  | 0.65     | 0.06  |
|            | 0.33    | 0.04  | 0.49     | 0.05  |
| Mean       | 0.37    | 0.04  | 0.61     | 0.06  |
| SD         | 0.06    |       | 0.11     |       |
| NS19       | 0.38    | 0.04  | 0.64     | 0.06  |
|            | 0.31    | 0.04  | 0.50     | 0.06  |
|            | 0.33    | 0.04  | 0.49     | 0.05  |
| Mean       | 0.31    | 0.04  | 0.52     | 0.06  |
| SD         | 0.08    |       | 0.08     |       |

*Repeat data on the yield of chromosomal aberrations in male volunteers after HDR irradiation and sampled over a period of 4 years. Calculated mean (mean) and standard deviation (SD) are listed.

**Table 2. Initial study: LDR irradiation data.***

| Identifier | Dic/cell | Error | Tab/cell | Error |
|------------|---------|-------|----------|-------|
| NS12       | 0.18    | 0.03  | 0.26     | 0.04  |
|            | 0.23    | 0.03  | 0.38     | 0.04  |
|            | 0.39    | 0.04  | 0.42     | 0.05  |
| Mean       | 0.26    | 0.04  | 0.53     | 0.05  |
| SD         | 0.09    |       | 0.44     | 0.11  |
| NS18       | 0.13    | 0.03  | 0.29     | 0.04  |
|            | 0.30    | 0.04  | 0.57     | 0.05  |
|            | 0.42    | 0.05  | 0.63     | 0.06  |
| Mean       | 0.33    | 0.04  | 0.53     | 0.05  |
| SD         | 0.07    |       | 0.53     | 0.05  |
| NS19       | 0.34    | 0.04  | 0.49     | 0.05  |
|            | 0.20    | 0.04  | 0.67     | 0.06  |
|            | 0.32    | 0.03  | 0.28     | 0.04  |
| Mean       | 0.25    | 0.03  | 0.38     | 0.09  |
| SD         | 0.06    |       | 0.09     |       |
| NS19       | 0.34    | 0.04  | 0.49     | 0.05  |
|            | 0.10    | 0.02  | 0.23     | 0.03  |
|            | 0.05    | 0.03  | 0.53     | 0.06  |
| Mean       | 0.17    | 0.03  | 0.33     | 0.05  |
| SD         | 0.04    |       | 0.11     |       |

*Repeat data on the yield of chromosomal aberrations in male volunteers after LDR irradiation and sampled over a period of 4 years. Calculated mean (mean) and standard deviation (SD) are listed.
HORMONAL EFFECTS ON RADIOSensitivity

Male donors sampled over a 4-year period. The age range was 30 to 43 years at the end of the sampling time.

Lymphocyte cultures were assayed for aberration levels after HDR and LDR treatment. A small number of samples were lost; thus, there was only one sample for volunteers numbered NS04, NS17, NS30, NS35, NS38, and NS47. Figure 1A,B shows the levels of TAb/cell in both male and female donors after HDR and LDR irradiation, respectively, whereas Table 3 presents a simple statistical summary of the data from the main study.

Analysis of variance (ANOVA) shown in Table 4 indicates that a highly significant difference ($p = 0.006$) between males and females was found following HDR irradiation; no significant difference between the sexes was found after LDR irradiation.

Exposure of male blood to final concentration of 3, 23, and 46 pg/ml progesterone increased the yield of dic/cell from 0.35 to a mean of 0.58, whereas the yield
Table 3. Statistical summary of radiosensitivity data from the main study.

|                | DIC/cell          | TAb/cell          |
|----------------|-------------------|-------------------|
|                | Male   | Female | Total | Male   | Female | Total |
| HDR            |        |        |       |        |        |       |
| Mean           | 0.38   | 0.42   | 0.40  | 0.61   | 0.71   | 0.66  |
| Standard error | 0.01   | 0.02   | 0.01  | 0.02   | 0.03   | 0.02  |
| Range          | 0.29   | 0.97   | 0.98  | 0.49   | 1.18   | 1.18  |
| Minimum value  | 0.2    | 0.21   | 0.2   | 0.39   | 0.35   | 0.35  |
| Maximum value  | 0.49   | 1.18   | 1.18  | 0.88   | 1.53   | 1.53  |
| HDR            |        |        |       |        |        |       |
| Mean           | 0.28   | 0.30   | 0.29  | 0.45   | 0.48   | 0.47  |
| Standard error | 0.01   | 0.01   | 0.01  | 0.01   | 0.02   | 0.01  |
| Range          | 0.31   | 0.55   | 0.55  | 0.43   | 0.76   | 0.76  |
| Minimum value  | 0.1    | 0.1    | 0.1   | 0.23   | 0.19   | 0.19  |
| Maximum value  | 0.41   | 0.65   | 0.65  | 0.66   | 0.95   | 0.95  |

*Standard error, range, minimum value, and maximum value are calculated for both HDR- and LDR-irradiated samples, for DIC/cell and TAb/cell and for males, females, and pooled (total) data.

Table 4. Results for analysis of variance (ANOVA) of radiosensitivity data from the main study.

|                | DIC+R | TAb    |
|----------------|-------|--------|
|                | HDR   | LDR    |
| Summary        | Count | Sum    | Average | Variance | Count | Sum    | Average | Variance |
| Males          | 48    | 18.19  | 0.38    | 0.004    | 48    | 29.49  | 0.61    | 0.011    |
| Females        | 46    | 19.43  | 0.42    | 0.023    | 46    | 32.59  | 0.71    | 0.043    |
| ANOVA          | DF    | Sum    | MS     | Vr     | p     | DF    | Sum    | MS     | Vr     | p     |
| Males          | Df    | 0.04   | 0.044  | 3.3    | 0.07  | 1     | 0.21   | 0.21   | 7.8    | 0.006 |
| Females        | 92    | 1.23   | 0.013  |        |       | 92    | 2.46   | 0.03   |        |       |

Abbreviations: DF, degrees of freedom; SS, sum of squares; MS, mean square; Vr, variance ratio; between, between groups; within, within group. *Calculated for both HDR- and LDR-irradiated samples, for DIC/cell and TAb/cell and for males, females.

Discussion

The initial 4-year study was conducted to establish the range and contribution of interexperimental variation to individual radiosensitivity, as measured by chromosome aberration induction. Statistical analysis of the variation in radiosensitivity observed among the male volunteers revealed that in both the initial study and the main study no true differences in individual sensitivity could be detected, i.e., any apparent variations were due to experimental uncertainty.

However, among the females, the statistical analysis revealed highly significant differences in sensitivity between female volunteers after LDR irradiation. This was significant at the 1% level for dicentrics + rings and at the 0.6% level for total aberrations. The variation in female radiosensitivity after HDR irradiation and analysis of total aberrations was found to be significantly greater than in males under comparable conditions. Age and environmental exposure to alcohol, cigarette smoking, occupational radiation exposure, prescribed drugs, and chemicals had no apparent effect on the levels of aberrations observed after either HDR or LDR exposure.

From the data presented in Figure 1A, B, there is no distinction between normal

Figure 2. Yield of DIC/cell and TAb/cell after treatment with various concentrations of 4-pregnen-3,20-dione (progesterone). Progesterone was added to samples of male blood 2 hr before irradiation.
and sensitive individuals as there is a wide range of sensitivities within the groups of individuals sampled. The larger range observed in the female samples was also accompanied by a greater degree of variation among samples. This implies that the factor enhancing female sensitivity is transitory, i.e., modulated response and not a genetic predisposition to radiosensitivity. Evidence suggesting that hormones play a role in modifying the radio-response of females can be seen upon closer inspection of the database. One female volunteer (NS33, data not shown) was taking conjugated estrogens without progesterone throughout this experiment, and both samples exhibited normal levels of gaps or breaks. After irradiation, another female volunteer (NS37) exhibited chromosomal yields similar to the mean for all female volunteers. However, 1 month after providing this blood sample, the volunteer commenced hormone replacement therapy. The repeat sample was taken 18 weeks after the first sample during a period when high progesterone doses were being taken with estrogens. The results showed a marked increase in chromosome gaps and breaks, from an initial 0.72 to 1.06 TAb/cell, representing a 68% increase. In both these examples, no increases in chromosomal aberration levels were noted in the control cultures. These data suggest that the female hormonal cycle could be contributing to the variation seen in female radiosensitivity, which implies that one possible cause could be hormonal changes.

An experiment on male blood demonstrated clearly that even small amounts of progesterone can alter the radio-response as measured by chromosome aberrations. A number of studies on hormonal effects have been conducted. Several studies detail chromosomal fragility, breakage, and sister chromatid exchanges resulting from exposure to hormones. Various estrogens have been shown to generate significant increases in chromatid gaps (14). Recent evidence from both in vivo and in vitro studies strongly suggests that estrogens are epigenotoxic carcinogens, i.e., they do not act directly as mutagenic or DNA-damaging agents but cause heritable changes by an unknown alternative mechanism (15). Progesterones also have similar effects, causing excess chromosomal gaps and breaks (16), and a synergistic effect was seen among progesterone, estrogen, and human chorionic gonadotrophin. Both prolactin and follicle-stimulating hormone produce excess chromosomal gaps and breaks (17,18). These reports clearly demonstrate that a variety of female hormones may play a role in modifying or enhancing DNA damage. Figure 3 demonstrates that as the level of progesterone increased in this female volunteer, so the chromosomal aberration level also rose. It is also of interest to note that the cellular effect of progesterone continued after the serum level returned to normal.

**Conclusion**

Two main conclusions can be drawn from this study. First, there are no statistically significant differences in radiosensitivity among males. What variation there is can be accounted for by intraexperimental errors. Second, the data suggest that variations in radiosensitivity among females after HDR irradiation is attributable to hormonal status.

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