Mitotic non-disjunction as a mechanism for in vivo aneuploidy induction by X-rays in primary human cells

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A collaborative study of three laboratories compared the induction of aneuploidy by X-rays in human lymphocytes and fibroblasts. The induction of non-disjunction versus chromosome loss by X-rays was investigated using a variety of aneuploidy detection methods. Chromosome loss was determined by fluorescence in situ hybridization (FISH) with pan-centromeric probes in cytochalasin-B-blocked binucleated cells. Chromosome non-disjunction was estimated by FISH with chromosome-specific centromeric probes in binucleated interphase cells. Chromosomes were counted in parallel in lymphocyte metaphase cells; chromosome counts of the whole karyotype and counts of chromosomes 2 and 8 using chromosome paints. A major observation in spontaneous non-disjunction frequencies concerned the clear difference in frequencies observed between the two painted chromosomes in the same primary cells. When cells were irradiated elevated frequencies were observed for all the different cytogenetic endpoints. Although only a small number of the micronuclei were positive for the centromeric signal and presumably contained whole chromosomes, the absolute number %C+ increased with dose. Higher rates of non-disjunction were found for irradiated cells; in fibroblasts a statistically significant increase was observed at a dose of 0.5 Gy. The detection of hyperdiploidy by means of chromosome counts and chromosome painting revealed an increase from doses of 1 Gy and higher. Comparison of the different methods detecting different endpoints indicates that non-disjunction may be an important mechanism leading to spontaneous and X-ray-induced aneuploidy. The relative radiosensitivity of aneuploidy induction was compared in two types of primary human cells—lymphocytes and fibroblasts. For chromosome loss both cell types showed similar results, whereas for non-disjunction fibroblasts seemed to be more sensitive. However, these differences may reflect a different sensitivity in the scoring methods used.

Introduction

The present work has addressed the question of whether ionizing radiation is capable of inducing aneuploidy in primary human cells. Radiation-induced aneuploidy has been reported in somatic cells, both in vitro and in vivo. Uchida et al. (1975) demonstrated a 4-fold increase in the frequency of hyperdiploid cells when in vitro cultures of human lymphocytes were exposed to low doses of γ-rays. In cultured mammalian cells low doses of X-rays resulted in the dislocation of chromosomes from the mitotic spindle (Parry et al., 1985). It has also been observed that in various cell lines a small number of radiation-induced micronuclei (MN) seem to contain whole chromosomes, as demonstrated by kinetochoore staining in MN (Nüsse et al., 1987; Degrassi and Tanzarella, 1988; Thomson and Perry, 1988; Eastmond and Tucker, 1989; Fenech and Morley, 1989; Gudi et al., 1990, Kirshna et al., 1992) or by in situ hybridization with a centromeric probe (Miller et al., 1992; Salassidis et al., 1992; Farooqi et al., 1993). More recently the induction of non-disjunction in vivo by low doses of X-ray was demonstrated by in situ hybridization in a transgenic mouse model (Boei and Natarajan, 1996) containing six marker chromosomes. In the follow-up study of radiation victims of the Goiana (Natarajan et al., 1991) and Chernobyl accidents (Natarajan et al., 1994) high frequencies of aneuploid cells were detected in cultured lymphocytes.

Within the framework of an EC research programme (Radiation Protection Research Action), our three laboratories have investigated the induction of non-disjunction and chromosome loss by X-rays (0.125–5.88 Gy) in human primary lymphocytes and fibroblasts. The potential events which may lead to aneuploidy during mitotic cell division are illustrated in Figure 1. Chromosome loss was determined by fluorescence in situ hybridization (FISH) with pan-centromeric probes in cytochalasin-B-blocked binucleates (Brussels on lymphocytes; Rome on fibroblasts). Chromosome non-disjunction was estimated by FISH with chromosome-specific centromeric probes in binucleated interphase cells (chromosomes 1 and 17 in Brussels; chromosomes 7 and 11 in Rome); chromosomes were counted in parallel on lymphocyte metaphase cells, chromosome counts of the whole karyotype (Brussels) and counts of chromosomes 2 and 8 using chromosome paints (Swansea).

Materials and methods

Cell cultures and irradiation protocol

Brussels. Human venous blood was collected from a healthy female donor (25 years) in heparin-containing vacutainers (Becton Dickinson, Lincoln Park, NJ). Lymphocyte cultures containing 0.4 ml whole blood and 4.9 ml Ham's F-10 medium (Gibco) supplemented with 15% fetal calf serum (Gibco) and 2% phytohaemagglutinin (PHA 16; Wellcome Diagnostic, UK) were incubated at 37°C for 74 or 78 h. For the preparation of metaphase chromosomes RPMI medium (Gibco) containing 1% bromodeoxyuridine (Serva) was used instead of Ham's F-10 medium in order to score chromosomes in their second division. The cells were exposed to 0.5, 1 and 2 Gy of 250 kVp X-rays (250 kV, 15 mA, 0.1 mm Cu filter) at a dose rate of 1 Gy/min during the G1 and the G2 stages of the cell cycle, here 20 and 42 h respectively after starting the cultivation. The time points of irradiation were chosen according to data from Buckton and Evans (1973) and Watt and Stephen (1986). No further analyses were performed.

Rome. Human lung primary fibroblasts (MRC-5) were maintained in minimal essential medium (Bio-Whitaker) supplemented with 10% fetal calf serum (Biological Industries) and antibiotics (penicillin and streptomycin; Hyclone) at 37°C in a humidified atmosphere containing 5% CO2 in air and irradiated with 0.125, 0.25, 0.50 and 1 Gy of X-rays at a dose rate of 0.70 Gy/min using a Gifardoni apparatus (250 kV, 6 mA, 0.2 mm Cu filter).
Swansea. Heparinized whole blood samples from healthy female donors (25 years) were irradiated with 0.74, 1.47, 2.94 and 5.88 Gy of X-rays at a dose rate of 1.47 Gy/min delivered by a GEC 50 K V X-ray source (50 kV, 20 mA, 1 mm Berullium filter). Cultures were grown at 37°C under a 3% CO2 atmosphere in RPMI 1640 medium (Gibico) supplemented with 20% fetal calf serum (Gibico) and 1% PHA.

Lymphocyte preparation

Brussels. Interphase analysis with cytochalasin-B block: after 44 h incubation at 37°C, cytochalasin-B (Sigma; stock solution 2 mg/ml in dimethylsulphoxide; final concentration diluted in Ham's F-10 medium) was added at a final concentration of 6 µg/ml (Van Hummel and Kirsch-Volders, 1990). The cells were harvested at different sampling times (74 and 78 h). Cells were subjected to a cold hypotonic treatment (0.075 M KCl), immediately centrifuged and fixed three times with fixative (methanol:acetic acid, 3:1). The fixed cells were dropped onto slides using Pasteur pipettes, air dried and stored at −20°C.

Metaphase analysis: colcemid (Gibico) was added to the cultures 1 h prior to fixation. Cells were given a hypotonic treatment (15 min, 37°C, 0.075 M KCl) and fixed three times in 3:1 methanol:acetic acid. The slides were stained for 10 min with Hoechst 33258 (Sigma) and 5% Giemsa (Merek) in Sterron buffer (pH 6.8).

Swansea. Metaphase analysis: following irradiation cultures were incubated for 96 h and colcemid (0.2 µg/ml) added for the last 3 h to collect metaphases. Cells were stained, restained, given a brief (10 min) hypotonic treatment (37°C, 0.075 M KCl) and fixed three times in 3:1 methanol:acetic acid. Fixed cells were dropped onto clean, polished slides, allowed to dry and stored at −20°C.

FISH

Brussels. FISH using a pancentromeric probe: the 30 nucleotide oligomer, shown to hybrdize to the conserved region of the α-satellite DNA present at the centromeres of all human chromosomes (Meyne et al., 1989), was used. This 30 oligonucleotide (50-αAlICen: Synthetic Oligomer α-All Centromeres) was synthesized with Gene Assembler Plus® (Pharmacia). The probe was 3′-end-labelled by terminal deoxynucleotidyl transferase (Gibco, Bethesda, MD) with biotin-16-4dUTP (Boehringer Mannheim). The cells were pretreated with pepsin (Sigma) (0.005% in 10 mM HCl) for 5 min at 37°C. The probe was diluted in an oligonucleotide hybridization buffer (25% deionized formamide, 2X SSC and 50 mM phosphate buffer, pH 7.0). Cells and probe were denatured simultaneously on a hot plate at 80°C for 3-4 min. Following overnight hybridization, immunofluorescence detection of the probe was performed by means of avidin-fluorescein isothiocyanate (FITC) and biotinylated goat anti-avidin (Vector Laboratories, Burlingame, CA). After dehydration in ethanol series, the slides were counterstained with propidium iodide (5 µg/ml) and p-phenylenediamine antifade solution (PPD) (Sigma, St Louis, MO) (1% PPD diluted in an oligonucleotide hybridization buffer (25% deionized formamide, 2X SSC and 50 mM phosphate buffer, pH 7.0). Cells and probe were denatured simultaneously on a hot plate at 80°C for 3-4 min. Following overnight hybridization, immunofluorescence detection of the probe was performed by means of avidin–fluorescein isothiocyanate (FITC) and biotinylated goat anti-avidin (Vector Laboratories, Burlingame, CA). After dehydration in ethanol series, the slides were counterstained with propidium iodide (5 µg/ml) and p-phenylenediamine antifade solution (PPD) (Sigma, St Louis, MO) (1% PPD diluted in glycerol).

FISH with chromosome-specific centromeric probes for chromosomes 1 and 17; probes for centromeric regions of chromosomes 1 (pUC 1.77) and 17 (D17Z1) were used. The probes were labelled by nick translation according to the instructions of the supplier (Life Technologies BRL). FISH was performed as described by Pinkel et al. (1986) with some modifications. Slides were treated with RNase (0.1 mg/ml in 2X SSC for 1 h at 37°C) and pepsin (Sigma) (0.005% in 10 mM HCl for 3 min at 37°C). The slides were denatured in 70% formamide/2X SSC for 2 min and dehydrated with ethanol (50, 75 and 100%). The slides and probe were denatured 3 min at 75°C. After dehydration the slides were washed with 60% formamide in 2X SSC at 45°C. The detection of the biotin-labelled probe was performed by means of avidin–FITC and biotinylated anti-avidin antibodies; the digoxigenin-labelled probe was detected using a mouse anti-digoxigenin antibody (Boehringer Mannheim) followed by a Texas red isothiocyanate (TRITC)-conjugated goat anti-mouse antibody (Boehringer Mannheim) and rabbit anti-goat FITC (Sigma); and (ii) avidin–Texas Red (Vector Laboratories) followed by biotinylated anti-avidin and avidin–Texas Red respectively. All slides were counterstained with DAPI (Sigma). Slides were mounted in Vectashield antifade.

Criteria for scoring

Brussels. MN were identified according to the criteria of Heddle (1973) with some modifications. They were classified as having less than a third of the diameter of either macronucleus and were sufficiently separated from them. For each culture 1000 binucleated lymphocytes (CB) were analysed for the presence of one, two or more MN.

For the FISH analysis the preparations were examined with a Leitz Dialux 20 fluorescence microscope equipped with a filter (Leitz, excitation at 450–490 nm and emission at 515 nm) to visualize the fluorescein-labelleed probe and the orange–red propidium iodide-stained nuclei. The specificity of the probes was evaluated using metaphase cells. The MN in CB were examined for the presence of one or more spots for the centromeric oligonucleotide probe and were classified as centromere-positive (C+) or centromere-negative (C−). For the chromosome-specific probes macronuclei were examined for the number of spots for each probe. Validation of the technique was carried out by analysing signals on metaphase and interphase nuclei. The following criteria for evaluating FISH signals were taken into account: the FISH signals should have more or less the same homogeneous fluorescence intensity; spots and nuclei should not overlap; and minor spots should not be counted (Devilee et al., 1988; Hopman et al., 1990; Arnoldus et al., 1991; Poddige et al., 1992; Segers et al., 1995). If one spot occurs in 10–20% of the total number of nuclei counted this should be ascribed to technical imperfections. A percentage of at least 80% of two spots should be observed in normal cells.

Metaphases in second division were scored for numerical chromosome aberrations. Metaphases with ≥2 chromosomes missing or extra were not included.

Rome. For each experimental point, a minimum of 1000 binucleated cells were analysed on coded slides for MN induction. Only MN not exceeding 1/3 of the main nucleus diameter, not overlapping the main nucleus and with distinct borders were included in the scoring.

All experiments with ‘all centromere’ cocktail probe the MN were located using a Zeiss Axioscope microscope with UV light and successively classified for the presence or absence of centromeric DNA, C+ or C− under blue violet illumination.

For the specific chromosome probe ultraviolet excitation was used to locate binucleated cells and hybridization signals were analysed using a double-band pass filter (Zeiss). To restrict the scoring to the first mitosis after treatment and to exclude technical artefacts, only binucleated cells with the correct number of hybridization signals were analysed. Scoring was performed taking into account signals with the same homogeneous fluorescence intensity. If in some part of a slide >2 nuclei were observed which contained no hybridization regions, that portion of the slide was considered inadequately hybridized and the nuclei were eliminated from scoring (Eastmond and Pinkel, 1990).

Swansea. The slides were visualised using an Olympus fluorescence microscope equipped with a triple-band pass filter to allow the simultaneous observation of both labelled chromosomes and counterstain. The images of the FISH-stained chromosomes were captured on a Perceptive Instruments.
Results

Centromere-positive MN as a measure for chromosome loss in cytochalasin-B-blocked lymphocytes and fibroblasts

The frequencies of MN and C+ MN were scored in human lymphocytes irradiated in either the G1 or G2 phase of the cell cycle (Table I). The time points of irradiation were chosen according to data from Buckton and Evans (1973) and Watt and Stephen (1986). Since ionizing radiation may cause a mitotic delay, lymphocytes were fixed at different sampling times (after 74 or 78 h of subcultivation) to collect most of the damaged cells, whether delayed or not. As expected from ionizing radiation, the majority of MN were C- (80-90%); however, an increase in absolute number of centromere positive MN (%C+) was observed after X-ray exposure. The increase was more pronounced for cells irradiated during the G2 phase than for those irradiated during the G1 phase. The increase was dose-dependent for G1 (r² = 0.976, P = 0.0121 (74 h) and r² = 0.952, P = 0.0241 (78 h)); no clear dose effect was observed for G2 cells (r² = 0.898, P = 0.0526 (74 h) and r² = 0.835, P = 0.2661 (78 h)).

In human primary fibroblasts arrested 24 h after irradiation, a dose-dependent increase in %C+ (r² = 0.958, P = 0.0038) was also observed (Table I). Moreover, the results obtained for the induction of %C+ were quite similar for both cell types.

Table II gives the number of binucleates analysed and the frequencies of unequal distribution of the four homologues between the two daughter nuclei, and the percentage of non-disjunction for two chromosomes (%ND) after FISH labelling with two chromosome specific centromeric probes. The targeted chromosomes were respectively chromosomes 1 and 17 in lymphocytes and chromosomes 7 and 11 in fibroblasts. The results obtained appeared to be chromosome-dependent, both for the spontaneous background and for radiation-induced non-disjunction.

In lymphocytes, chromosome 1 was more frequently involved in spontaneous non-disjunction than chromosome 17. However, after irradiation chromosome 17 showed an increase in %ND which was not observed for chromosome 1; moreover, cells that were in the G2 phase at the time of exposure showed a higher non-disjunction rate for chromosome 17 than those that were in G1. No dose response was observed for the overall frequency of non-disjunction or when the frequencies of the individual chromosomes were considered separately.

For fibroblasts, chromosome 11 was more frequently involved than chromosome 7 in both spontaneously and radiation-induced non-disjunction. After irradiation with 0.5 Gy a statistically significant (P = 0.0125) increase in the frequency of non-disjunction was seen.

The frequencies of non-disjunction observed in fibroblasts were higher than those in lymphocytes.

Aneuploidy frequencies in second metaphase lymphocytes

Chromosome counting was performed either on Giemsa-stained metaphases from lymphocytes irradiated in G1 or G2, or on metaphases from G0 irradiated cells painted with probes for chromosomes 2 and 8 (Table III).

Hyperdiploidy was increased in cells irradiated with 1 Gy or more; moreover the increase was found to be dose-dependent in the G0 (r² = 0.875, P = 0.0195) and G2 irradiated cells.
Table II. Frequencies of chromosome non-disjunction by chromosome-specific centromere probing on interphase in cytochalasin-B-blocked binucleates

<table>
<thead>
<tr>
<th>Brussels Human lymphocytes</th>
<th>number of spots</th>
<th>1+17</th>
<th>1+17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixation at 78h</td>
<td>Dose (Gy)</td>
<td>nCB</td>
<td>(2+2)CB</td>
</tr>
<tr>
<td></td>
<td>0.0</td>
<td>490</td>
<td>482</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>571</td>
<td>562</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>636</td>
<td>629</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>624</td>
<td>612</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Roma Human fibroblasts</th>
<th>number of spots</th>
<th>7+11</th>
<th>7+11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixation 24h after irradiation and addition of cyto-B</td>
<td>Dose (Gy)</td>
<td>nCB</td>
<td>(2+2)CB</td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td>1500</td>
<td>1495</td>
</tr>
<tr>
<td></td>
<td>0.50</td>
<td>1000</td>
<td>992</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1500</td>
<td>1493</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>1500</td>
<td>1489</td>
</tr>
</tbody>
</table>

%ND: percentage of non-disjunction in binucleates (CB) is the number of binucleates with uneven (but < 4) distribution of spots for either chromosomes.

*Chi-square test: P < 0.05.

Table III. Aneuploidy frequencies in second metaphase lymphocytes

<table>
<thead>
<tr>
<th>Brussels chromosome counts of whole metaphase on human lymphocytes G1 and G2 irradiation; dose rate 1 Gy/min; fixation at 78h</th>
<th>n cells</th>
<th>%hypodiploid</th>
<th>%hyperdiploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (Gy)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>200</td>
<td>15.5</td>
<td>3.5</td>
</tr>
<tr>
<td>0.50</td>
<td>200</td>
<td>10.0</td>
<td>3.0</td>
</tr>
<tr>
<td>1.00</td>
<td>199</td>
<td>15.0</td>
<td>6.5</td>
</tr>
<tr>
<td>2.00</td>
<td>200</td>
<td>35.0</td>
<td>6.5</td>
</tr>
<tr>
<td>0.50</td>
<td>200</td>
<td>10.0</td>
<td>4.5</td>
</tr>
<tr>
<td>1.00</td>
<td>200</td>
<td>13.5</td>
<td>7.0</td>
</tr>
<tr>
<td>2.00</td>
<td>112</td>
<td>13.8</td>
<td>9.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Swansea chromosome counts of specific chromosomes using whole chromosome paints in metaphase human lymphocytes G0 irradiation; dose rate 1.47 Gy/min; fixation at 96h</th>
<th>n cells</th>
<th>Chromosome 2</th>
<th>%hypodiploid</th>
<th>%hyperdiploid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (Gy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>350</td>
<td>3.2</td>
<td>0.0</td>
<td>4.0</td>
</tr>
<tr>
<td>0.74</td>
<td>200</td>
<td>2.5</td>
<td>0.0</td>
<td>4.0</td>
</tr>
<tr>
<td>1.47</td>
<td>200</td>
<td>7.0</td>
<td>1.0</td>
<td>7.0</td>
</tr>
<tr>
<td>2.94</td>
<td>200</td>
<td>4.5</td>
<td>1.0</td>
<td>7.0</td>
</tr>
<tr>
<td>5.88</td>
<td>128</td>
<td>3.1</td>
<td>2.3</td>
<td>6.3</td>
</tr>
</tbody>
</table>

$r^2 = 0.971$, $P = 0.0144$. In contrast, hypodiploidy did not show a consistent dose-dependent increase in either protocol.

Discussion

In this study the induction of chromosome malsegregation by X-rays was compared in two types of primary human cells—lymphocytes and fibroblasts—and evaluated at three different cytogenetic endpoint levels: chromosome loss measured in MN, chromosome non-disjunction and hypo/hyperdiploidy in second metaphases.

X-ray-induced chromosome loss, as determined by the micronucleus assay with a pan-centromeric probe, was demonstrated in both cell types. A small fraction of the MN (10-20%) was positive for the FISH signal, which indicates that they most probably contain a whole chromosome as a result of chromosome lagging. Where data are available for both lymphocytes and fibroblasts, a good agreement was found...
Mitotic non-disjunction and aneuploidy induction

Fig. 1. Events leading to aneuploidy during mitotic cell division. During mitotic cell division aneuploidy may occur by a variety of malsegregation events, the most important of which are: (i) chromosome loss, e.g. when a chromosome is dislocated from the mitotic spindle. Such a process will lead to the formation of 2n and 2n - 1 progeny cells. The dislocated chromosome may lead to the formation of a micronucleus. This event may be detected by the observation of whole chromosome containing MN in interphase cells or as hypodiploid metaphase cells. (ii) Mitotic non-disjunction, e.g. when both copies of a chromosome segregate to the same pole of a dividing cell results in 2n + 1 and 2n - 1 progeny cells. Non-disjunction may be detected by the observation of hypodiploid and hyperdiploid interphases and metaphases in progeny cells.

between the results obtained for both cell types. A dose-dependent increase in %C + was found for G1 but not for G2 cells. The variation in cell cycle sensitivity suggests that different targets might be involved in chromosome loss during the different stages of the cell cycle.

As far as chromosome non-disjunction is concerned, the results within and between both cell types showed limited agreement. The first major observation about the spontaneous non-disjunction frequencies concerns the clear difference observed between two chromosomes in the same primary cells. In lymphocytes a higher spontaneous non-disjunction was observed for chromosome 1 (1.6%) and a lower one for chromosome 17 (0%); in fibroblasts a higher frequency was found for chromosome 11 (0.7%) than for chromosome 7 (0.3%). One might speculate about the causes of these differences. An important factor is that non-disjunction might be a nonrandom phenomenon. Certain chromosomes might be involved more frequently than others. Early studies on the identification of the chromosomes lost in hypodiploid leukocytes showed that chromosome loss depends on a variety of factors such as chromosome size, age and sex (Neurath et al., 1970; Fitzgerald and McEwan, 1977). In addition, the differences observed might be due to some viable aneuploid types having different selective disadvantages.

Although non-disjunction is probably not random, one might speculate about the total non-disjunction frequency derived from the non-disjunction observed for the two chromosomes. Assuming that non-disjunction is randomly distributed over the different chromosomes, the 'true' non-disjunction frequency may be extrapolated from these data using a correction factor of 23/2. In both assays a high background frequency for the whole genome was calculated—18.4% for lymphocytes and 11.5% for fibroblasts. These data agree with previous estimations from chromosome counts which calculated frequencies of 6.66% hyperdiploid and 17.91% hypodiploid in human lymphocytes (Verschaeve et al., 1978). However, one should also take into account that in these earlier studies a fraction of the hypodiploid cells might be due to technical artefacts.

The variability in the response found for the frequencies in irradiated cells might be explained by the fact that in both experiments different chromosomes, apparently with different sensitivity towards non-disjunction, were chosen as targets. However, it might also reflect a difference in sensitivity towards the cell type in question.

The rather high rates of non-disjunction in controls and irradiated cells may be innate to the methods used. In contrast to the micronucleus assay, which detects only chromosome loss, and to chromosome counting, which only allows scoring of hyperdiploidy in second metaphase, interphase analysis using chromosome specific probes detects both hyper- and hypodiploidy during the first interphase following the aneugenic event. On the other hand, there are indications that higher rates of chromosome maldistribution might appear after longer fixation times possibly as a result of multipolar mitoses occurring in binucleated cells which escape the cytochalasin B block (Zijno et al., 1994). The higher rates of non-disjunction could be partially due to the effect of cytochalasin B. It has been reported that cytochalasin B increases the spontaneous micronucleus rates and leads to abnormal anaphase figures.
Fig. 2. Estimation of the relative contribution of chromosome loss versus non-disjunction to aneuploidy in human lymphocytes.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Chromosome loss</th>
<th>Non-disjunction</th>
<th>Aneuploidy</th>
<th>Hyperdiploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%C + MNCB*</td>
<td>%NDCB*</td>
<td>%aneuploidy</td>
<td>%hyper (FISH)*</td>
</tr>
<tr>
<td>0.00</td>
<td>0.7</td>
<td>18.4</td>
<td>7.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.50</td>
<td>1.9</td>
<td>18.4</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>0.74</td>
<td>3.7</td>
<td>17.3</td>
<td>13.0</td>
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</tr>
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<td>1.00</td>
<td>5.4</td>
<td>25.3</td>
<td>13.0</td>
<td>28.8</td>
</tr>
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<td>1.47</td>
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</tr>
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</tr>
<tr>
<td>2.94</td>
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<td></td>
</tr>
<tr>
<td>5.88</td>
<td></td>
<td></td>
<td></td>
<td>28.8</td>
</tr>
</tbody>
</table>

*Chromosome loss (Data Table I: Brussels, %C+, G1 irradiation, fixation after 78 h).

Total non-disjunction frequencies derived from the frequencies observed for two chromosomes (1 and 17) (Data Table II: Brussels, %ND, G1 irradiation, fixation after 78 h) using a correction factor of 23/2.

Aneuploidy; since hypoploidy could be due to technical artefacts, the total aneuploidy was considered to be equal to twice the hyperdiploidy frequency (Data Table III: Brussels, %hyperdiploid, G1 irradiation, fixation after 78 h).

Hyperdiploidy derived from the frequencies observed for the chromosomes 2 and 8 (Data Table III: Swansea).

(Lindholm et al., 1991; Norppa et al., 1993). The exact mechanisms of action of cytochalasin B are not yet clear. However, it can be assumed that, in the presence of cytochalasin B, the first nuclear division is slightly affected, subsequent divisions showing a higher number of errors as a mechanistically yet unknown consequence of the disruption of the cytoskeleton (Norppa et al., 1993).

The most relevant estimation for aneuploidy induction is, of course, the counting of chromosome gain versus chromosome loss in second metaphases following the treatment. As far as the lymphocytes are concerned, irradiation in G1 induced a more dose-dependent and slightly higher frequency of hyperdiploid metaphases. This suggests that both G1 and G2 cells were sensitive to the induction of chromosome malsegregation by X-rays and that different targets might be involved in X-ray-induced aneuploidy. One may indeed consider that microtubules might be preferential targets in G2 but that both in G1 and G2 DNA lesions in centromeres/telomeres may contribute to chromosome malsegregation. Irradiation in G0 and the scoring of painted chromosomes confirmed the dose-dependent increase of hyperdiploidy. The chromosome painting method, which is, of course, less time consuming, seems also more sensitive since the background frequency is very low; however, the differences in hyperdiploid frequencies between G0 and G1 or G2 cells might be due to either the scoring method or the cell phase differential sensitivity.

By comparing the frequencies of hyperdiploidy (Table III) with the frequencies of chromosome loss (Table I) and non-disjunction (Table II) one may estimate the relative contribution of chromosome loss versus non-disjunction to aneuploidy in human primary cells. In Figure 2 an attempt was made to quantify these events in lymphocytes irradiated in G1 (Brussels) and G0 (Swansea). Since hypodiploidy could in part be due to technical artefacts, the total aneuploidy was considered to be equal to twice the hyperdiploidy frequency. A correction factor (23/2) was applied to the hyperdiploidy data obtained with chromosome painting and to the frequencies of chromosome non-disjunction as well. Frequencies of chromosome loss were derived from the frequencies of centromere-positive MN per 1000 binucleates. These data should be treated with caution since ionizing radiation can break DNA at the centromeres and induce centromere-positive MN which are not the result of an aneugenic event. This comparison indicates that only a small fraction of the spontaneous and radiation-induced...
aneupeploly was detected using the micronucleus assay. Non-disjunction seems to be a far more important mechanism leading to spontaneous and radiation-induced aneupeploly. Higher frequencies of nondisjunction and hyperdiploidy were obtained using FISH techniques, chromosome-specific probing in interphase and chromosome painting in metaphase, compared with chromosome counting on Giemsa-stained metaphases. This suggests that in the classical cytogenetic test aneupeploly might have been systematiplcally underestimated and that molecular cytogenetic methods probably provide more sensitive evaluations of potential aneuploids.

On the basis of the observed results, one may hypothesize about the targets involved in X-ray-induced aneupeploly. The comparison of the results obtained by the different scoring methods indicates that X-ray-induced aneupeploly probably arises by means of interaction with several targets. As illustrated by the micronucleus assay, where higher frequencies of chromosome loss were observed in G1 phase, aneupeploly during the G1 phase might be preferentially attained by interaction with DNA targets. The higher levels of aneupeploly in G2 phase, as observed with chromosome-specific probing on interphases and chromosome counts on metaphases, on the other hand suggest that additional non-DNA targets might be involved at this stage of the cell cycle. Obviously, interactions with the spindle apparatus should be considered, but DNA targets at the level of both centromeres and the nuclear membrane attached telomeres should not be overlooked.

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References


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