No increase in radiation-induced chromosome aberration complexity detected by m-FISH after culture in the presence of 5'-bromodeoxyuridine

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Abstract

The thymidine analogue, 5'-bromodeoxyuridine (BrdU), is a known mutagen that is routinely introduced into culture media for subsequent Harlequin stain analysis and determination of cell cycle status. Previously, we examined the induction of chromosome aberrations in human peripheral blood lymphocytes (PBL) known to be in their 1st cell division following exposure to a low dose (0.5 Gy, average one α-particle per cell) of high-LET α-particles. We found complex chromosome aberrations to be characteristic of exposure to high-LET radiation and suggested the features of complex exchange to reflect qualitatively the spatial deposition of this densely ionising radiation. To exclude the possibility that BrdU addition post-irradiation influenced the complexity of chromosomal damage observed by m-FISH, the effect of increasing BrdU concentration on aberration complexity was investigated. Comparisons between BrdU concentration (0, 10, and 40 μM) and between sham- and α-particle irradiated PBL, were made both independently and in combination to enable discrimination between BrdU and high-LET radiation effects. Aberration type, size, complexity and completeness were assessed by m-FISH, and the relative progression through cell division was evaluated. We found no evidence of any qualitative difference in the complexity of damage as visualized by m-FISH but did observe an increase in the frequency of complex exchanges with increasing BrdU concentration indicative of altered cell cycle kinetics. The parameters measured here are consistent with findings from previous *in vitro* and *in vivo* work, indicating that each complex aberration visualised by m-FISH is characteristic of the structure of the high-LET α-particle track and the geometry of cell irradiated.

1. Introduction

Exposure to ionising radiation effectively results in the formation of chromosome exchange aberrations. The type and frequency of aberration induced is now well established to be dependent upon radiation quality, dose and cell type such that their occurrence can be exploited for biodosimetric purposes and also, for the mechanistic study of how radiation-induced chromosome aberrations may be formed . For most studies, such an assessment of initially induced damage relies on the identification of cells that are in their 1st cell division after exposure. This can be achieved through the incorporation of the thymidine analogue, 5'-bromodeoxyuridine (BrdU), during successive cell cycling and subsequent Harlequin staining of metaphase chromosomes. The principle of this technique considers the unineme structure of DNA and the fact that newly synthesised DNA will contain both thymidine and BrdU. Depending on which cell division the metaphase represents and, if beyond 1st division, which DNA template was used for strand synthesis, then each chromatid of each metaphase chromosome will be comprised of either single or double stranded BrdU-incorporated DNA. This can be visualised by fluorescent or Giemsa differential chromatid staining . Thus, metaphases in their 1st, 2nd or 3rd cell division after radiation exposure can be differentiated .

Complex chromosome aberrations, defined as any exchange that involves 3 or more breaks in 2 or more chromosomes , are commonly induced after exposure to low doses of high-LET α-particle radiation . Their occurrence and complexity have been related to be a direct consequence of the structure of the high-LET α-particle track and the number of different chromosome territories intersected by each individual particle . For many studies, BrdU is routinely added to the culture media approximately 1 hour post-G_0 irradiation. BrdU however, is thought to influence genetic stability when incorporated into newly synthesising DNA, altering higher

order conformation of DNA and resulting in local decondensation of chromatin and disruption of normal interactions with repair enzymes . Consequently the presence of BrdU in the culture media after irradiation, could sterically prevent, modify or delay the rejoining of the α-particle-induced breaks, if incorporated at damage sites during repair processing for example. Such modifications could, at least theoretically, be expressed by an increase in the complexity of aberration subsequently observed.

The aim of this study was to examine whether the complexity of aberration induced by α-particle irradiation was in any way influenced by the post-irradiation addition of BrdU. Using the technique of m-FISH, we found no difference between increasing concentrations of BrdU in the degree of α-particle-induced aberration complexity observed.

2. Materials and methods

2.1 Cell culture and irradiation

Whole blood was collected from healthy adult donors according to the guidelines issued by the Medical Research Council in *Responsibility in Investigations on Human Participants and Material and on Personal Information* (MRC Ethics series, November 1992). Peripheral blood lymphocytes (PBL) were isolated based on Ficoll density gradient centrifugation using sodium heparin Vacutainer tubes (Becton Dickinson, UK), and washed in Ca^{2+}/Mg^{2+} free phosphate buffered saline (PBS) (Sigma-Aldrich, UK). Pooled cells were seeded at $1x10^6$/ml in basic media (RPMI 1640, Dutch modification, (Invitrogen, UK)) containing 10% heat-inactivated foetal

bovine serum (FBS) (Invitrogen, UK), 100 IU/ml penicillin, 100 μg/ml streptomycin, 2mM L-glutamine, 2mM sodium pyruvate (Sigma-Aldrich, UK).

Following overnight incubation at 37°C, 5% CO_2, PBL were plated into Hostaphan dishes (35mm glass rings with 2.5μm thick replaceable Hostaphan (0.35 mg cm^{-2} polyethylene terephthalate; Hoechst) bases for α-particle irradiation ($2x10^6$ cells/dish). An 8 μl drop of cell suspension was placed on the base of each dish and overlaid with a CR39 plastic disc (poly-allyl diglycol carbonate - polymeric Solid State Nuclear Track Detector), resulting in the formation of a cell monolayer between the CR39 and Hostaphan base . PBL were exposed to a ^{238}Pu α-particle source and irradiated with a dose of 0.5 Gy (equivalent to a mean of 1 α-particle traversal/cell). The incident energy at the Hostaphan base was 3.26 MeV giving an LET of 121.4 keV/μm. Control samples were sham irradiated.

Within 1 hour of exposure, PBL were stimulated to divide in 4 ml volumes of fresh culture media (basic media plus 0.5 μg/ml purified phytohaemagglutinin (PHA: HA16, Bio-Stat Ltd, UK)) at a seeding density of $4x10^5$/ml. At the same time, 0, 10 or 40 μM (0, 1.25 or 2.5 μg/ml) 5'-bromodeoxyuridine (BrdU) (Sigma-Aldrich, UK) was added. PBL were incubated at 37°C, 5% CO_2 for a total of 50 hours, and harvested to obtain 1st division metaphase cells. Briefly, 5 μl/ml colcemid (Sigma, UK) was added for the final 1.5 hours of culture. Cells were then treated with KCl hypotonic (0.075M) at 37°C for 8 minutes prior to fixation in 3:1 methanol:acetic acid and storage at –20°C.

2.2 Multiplex FISH (m-FISH)

For m-FISH, slides were left at room temperature for 24 hours and aged by incubating in cell fixative for 1 hour (3:1 methanol:acetic acid), baking at 67°C for 20 minutes and immersing in acetone for 10 minutes. Hybridisation pretreatments were carried out at 37°C, consisting of 1 hours incubation in 100 µg/ml DNAse free RNAse (Sigma-Aldrich, UK) and 5-10 minutes digestion in pepsin (1:20x10^3 in 10 mM HCl) (Sigma-Aldrich, UK). Slides were rinsed twice in 2xSSC and PBS between pretreatments, and twice in PBS following pretreatments (all washes 5 minutes). Additional washes in 50 mM $MgCl_2$ in PBS, and 50 mM MgCl in PBS with 1% formaldehyde (5 minutes each) preceded slide dehydration through an ethanol series (70, 70, 90, 90, 100% ethanol for 2 minutes each).

For hybridisation, probe cocktail (24-colour paint Spectravision assay, Vysis Ltd UK) was denatured at 72ºC for 6 minutes. In parallel, metaphase chromosomes were denatured at 72°C for 3 minutes in 70% formaldehyde/2xSSC, dehydrated through an ethanol series (70, 90, 100% for 1 minute each), and air-dried. Cells and probe were then mixed and left to hybridise for 36-48 hours at 37ºC before being washed in 0.4xSSC/0.3% Igepal (Sigma-Aldrich, UK) for 1.5 minutes at 71ºC and 2xSSC/0.1% Igepal for 10 seconds at room temperature. DAPI III counterstain (Vysis Ltd, UK) was applied to air-dried slides, coverslips sealed and cells stored in the dark at -20ºC.

Metaphase chromosomes were visualized using a six-position Olympus BX51 fluorescence microscope containing individual filter sets for each component fluor of the Spectravision probe cocktail plus DAPI (Spectrum Gold, Spectrum Far-red, Spectrum Aqua, Spectrum Red and Spectrum Green). Digital images were captured for m-FISH using a charge-coupled device (CCD) camera (Photometrics Sensys CCD, UK) coupled to and driven by Genus (Applied Imaging, UK). In the first instance, cells were karyotyped and analysed by enhanced DAPI banding. Detailed

paint analysis was then performed by assessing paint coverage for each individual fluor down the length of each individual chromosome, using both the raw and processed images for each fluor channel. A cell was classified as being apparently normal if all 46 chromosomes were observed by this process, and subsequently confirmed by the Genus m-FISH assignment, to have their appropriate combinatorial paint composition down their entire length.

2.3 Classification of chromosome aberrations

Cytogenetic analysis by m-FISH was carried out on ~600 cells from 3 replicate experiments using coded slides to ensure unbiased data collection. Aberrations were categorized as being of chromatid or chromosome type. Among the latter, exchange aberrations involving 3 or more breaks in 2 or more chromosomes were classed as complex, rearrangements involving only 2 breaks in 1 or 2 chromosomes as simple, and unrestituted free ends not apparently involved in an exchange of material between chromosomes, as single chromosome breaks .

Complex exchanges were also categorised according to C/A/B , enabling assessment of their size and complexity based on the minimum number of rearranged Chromosomes, Arms and Breaks observed in each exchange. Evaluation of aberration 'completeness' was based on the presence/absence of free chromosome ends within each observed chromosomal rearrangement. Exchanges were designated as 'incomplete' if more than one chromosome end remained unrejoined, or as 'complete' when all of the free-ends had apparently successfully rejoined (for detail see).

2.4 Harlequin staining

Metaphase preparations for analysis of differential (Harlequin) staining were produced using a modified version of the fluorescence plus Giemsa (FPG) method . Briefly, slides of fixed PBL samples, kept in the dark and at room temperature for a minimum of 3 days, were immersed in Hoescht 33258 (20 μg/ml in dH_20, Sigma-Aldrich, UK) for 10 minutes, then submerged in a thin layer (~1mm) of 2xSSC during 25 minutes UV exposure (Philips TYP 57135 G 400 W lamp at ~12 inches). After washing in dH_2O (3 x 5 minutes), slides were air dried, stained with 3-6% Giemsa in pH 6.8 Sorenson's buffer for 5 minutes, and mounted with xylene (VWR International, UK). Based on differences in Giemsa binding to BrdU or thymidine incorporated DNA strands, cell division was classified according to the following sister chromatid staining patterns:

- In 1st division metaphase cells, one strand of each sister chromatid contains BrdU producing uniformly stained chromosomes.
- In 2nd division metaphase cells, sister chromatid pairs have a 2:1 BrdU content that produces differentially stained chromosomes with a characteristic harlequin (light:dark) appearance.
- 3rd division metaphase cells display a mix of 2nd division and 2:2 BrdU chromosome staining patterns.

From at least 100 metaphases analysed, the number of 1st, 2nd and 3rd division mitotic cells were scored for each PBL sample.

2.5 Mitotic Index

The Mitotic Index (MI) of each PBL sample was obtained using the following formula:

$$MI = \frac{\text{number of metaphase cells observed}}{\text{total number of cells scored}} \times 100$$

A minimum of 2000 cells were scored for each test to determine the proportion of cells in metaphase at the time of harvest.

2.6 Statistics

Data on complex aberration type and completeness, and mitotic indices were tested using the multi-sample equivalent of Fisher's exact test. Analysis of aberration size and complexity, as measured by the mean number of breaks and chromosomes observed, was tested using the Kruskal-Wallis test.

3. Results

In three independent experiments, peripheral blood lymphocytes (PBL) exposed to 0.5 Gy high-LET α-particles were stimulated to divide and cultured in medium supplemented with varying concentrations of 5'-bromodeoxyuridine (BrdU). A total of 821 metaphase cells were analysed by m-FISH following 50 hours in culture, and all observed chromosomal exchanges were categorised as simple or complex.

Complex rearrangements were further assessed based on measurements of completeness, size and complexity. No statistical interexperimental difference was observed for any of these endpoints, allowing data to be pooled.

3.1 Effect of BrdU on induction of chromosome exchanges

The frequency and type of chromosomal aberrations induced in PBL after exposure to either sham or high-LET α-particle radiation and cultured in the presence of 0, 10 or 40 μM BrdU, are shown in Table 1. For sham irradiation, the total exchange frequency ranged from 0.000 – 0.017 and is consistent with expected background levels .

A significant increase in the frequency of both simple and complex aberrations was observed in irradiated compared to sham-irradiated PBL, independent of BrdU concentration. The occurrence of simple exchanges increased from background to 0.069 ($p=0.014$), 0.044 ($p=0.041$) and 0.090 ($p=0.004$) and complex exchanges to 0.123, 0.109 and 0.193 ($p<0.0001$) for cells cultured in media containing 0, 10 and 40 μM BrdU, respectively, after high-LET α-particle irradiation (Table 1).

To investigate the effects of increasing BrdU concentration on the formation of high-LET α-particle induced chromosome aberrations, the total aberration frequency and the proportion of exchanges classified as either simple or complex (expressed as S:C ratio) were assessed. A significant increase in the percentage of cells damaged by α-particle irradiation was observed in the presence of 40 μM BrdU (23%) compared to either 0 or 10 μM BrdU (15% and 13%, $p=0.014$) (Table 1). This is paralleled by an increase in the frequency of complex aberrations (0.193 for 40 μM BrdU compared to 0.123 and 0.109 for 0 or 10 μM BrdU, respectively, $p=0.036$), but not simple

aberrations, which remain constant with varying BrdU concentration (p=0.220) (Table 1). Overall, no difference in S:C ratio was observed between α-particle irradiated PBL when cultured in the presence of 0, 10 or 40 μM BrdU (0.56, 0.41 and 0.46, respectively, p=0.805) (Table 1).

3.2 *Effect of BrdU on the complexity of α-particle-induced complex chromosome aberrations*

To assess the influence of BrdU in modifying the complexity of α-particle induced complex aberrations observed by m-FISH, the size of each rearrangement and its 'completeness' were measured. The minimum number of breaks and chromosomes involved in each complex exchange were resolved (for detail see) and their mean values compared for each concentration of BrdU. Using this classification, no significant difference in complex aberration size was seen. On average 3.28, 3.56 and 3.68 chromosomes (range 2-7 different chromosomes, p=0.590) and 5.04, 5.19 and 5.21 breaks (range 3-11 breaks, p=0.930) were involved in each individual complex exchange in 0, 10, and 40 μM BrdU samples, respectively. One complex aberration involving 8 chromosomes and 17 breaks was observed in the 40 μM BrdU sample, however the possibility that this aberration represents more than one independent rearrangement cannot be excluded .

Each complex chromosome aberration was then classified as 'incomplete' or 'complete' as previously described, based on the presence or absence of unrejoined chromosome break-ends respectively (for detail see). The proportion of 'completeness' did not significantly decrease in the presence of 10 or 40 μM BrdU (the fraction of total complex exchanges classified as complete was 0.486, p=0.560

and 0.380, p=1.000, respectively) compared to PBL cultured in the absence of BrdU (0.344) (Fig. 1). Overall, we found no evidence to suggest that aberration complexity was influenced by the presence of 40 μM BrdU in the culture media after high-LET α-particle irradiation when assessed by m-FISH.

3.3 Effect of BrdU on cell cycle progression

To investigate the influence of BrdU on rate of cell cycle progression following α-particle exposure, Harlequin analysis, for determination of metaphase cell division, was performed. The proportion of cells in metaphase at the time of harvest (mitotic index (MI)) was also assessed for PBL samples at each BrdU concentration.

For 10 and 40 μM BrdU, respectively, Harlequin staining showed 93% and 95% of the total metaphase cells analysed to be in their 1st cell division 50 hours after high-LET α-particle irradiation (200-300 cells analysed/sample), with the remaining fraction in 2nd division (Table 2). Similarly, 96% and 93% of metaphase cells sampled from sham-irradiated populations cultured in the presence of 10 and 40 μM BrdU, respectively, were in their 1st cell division at this time point. Sham and α-particle irradiated PBL cultured in the absence of BrdU were not Harlequin stained since the addition of BrdU is inherent for the technique.

Between 2,000 and 6,000 cells were counted to determine the MI for all sham and α-particle irradiated samples. For sham-irradiated PBL, MI decreased significantly with increasing BrdU concentration (from a mean of 1.8% in cultures lacking BrdU to 1.4% and 0.6% in samples containing 10 and 40 μM BrdU, respectively, p=0.015). The same trend, although not significant, was seen after exposure to high-LET α-

particles, where MI decreased from a mean of 1.4% in the controls or in the presence of 10 μM BrdU to 1% in the presence of 40 μM BrdU (Table 2).

3.4 Effect of BrdU on chromosome and chromatid breaks

The influence of BrdU on the incidence of other aberration types was assessed, focussing specifically on chromosome breaks (classified as all breaks apparently not involved in an exchange) and chromatid breaks. No significant difference was found in the frequency of chromosome breaks between sham and α-particle irradiated PBL after culture in varying concentrations of BrdU. Specifically, the chromosome break frequency was 0.025 and 0.020 (p=0.710) after culture in 0 μM, 0.000 and 0.036 in 10 μM (p=0.060) and 0.006 and 0.021 in 40 μM BrdU (p=0.330) for sham and α-particle irradiated cells, respectively (Table 1). No chromatid breaks were observed in sham-irradiated samples containing 0 or 10 μM BrdU and at 40 μM, the frequency of chromatid breaks (0.006) was no higher than background . However, α-particle exposed PBL cultured in the presence of 40 μM BrdU were observed to have a significantly increased frequency of chromatid breaks (0.034) compared to PBL cultured in 0 μM BrdU (0.000, p=0.012) and 10 μM BrdU (0.009, p=0.027) (Table 1).

4. Discussion

Previous studies have shown complex chromosome aberrations to be commonly induced in peripheral blood lymphocytes (PBL) after exposure to low doses of high-LET α-particle radiation . Further, their detailed analysis, using the technique of m-FISH, reveals this complexity to be qualitatively characteristic of individual high-LET

α-particle tracks and also, the geometry of cell nuclei traversed .

5'-bromodeoxyuridine (BrdU), a known mutagen, is routinely added to culture media following radiation exposure for subsequent Harlequin stain analysis and identification of 1st cell division mitoses . The purpose of this study therefore, is to examine whether the presence of BrdU in PBL culture media, when added after high-LET α-particle exposure, influences the complexity of chromosome aberrations that are initially observed by m-FISH.

Significantly increased levels of chromosomal damage were observed in PBL exposed to high-LET α-particles compared to sham-irradiated samples independent of BrdU concentration (up to 40 μM), demonstrating that BrdU alone does not produce chromosome exchange aberrations in PBL. Similar findings have been reported elsewhere. For example, no chromosome exchanges were observed above background in unirradiated PBL after 72 hours of culture in 40 μM BrdU , or at the higher concentration of 80 μM BrdU . Thus, the formation of chromosome exchanges in PBL in this study, and in particular complex exchanges, is dependent on exposure to high-LET α-particles.

A significant increase in the frequency of damaged cells was detected when PBL were exposed to high-LET α-particles and cultured in the presence of 40 μM BrdU. In parallel, an elevated frequency of complex exchanges was seen relative to those PBL cultured in lower concentrations of BrdU (0.123, 0.109 and 0.193 for PBL cultured in 0, 10 and 40 μM BrdU respectively) (Table 1). To assess whether this increased frequency was also associated with an increase in exchange complexity, the size of each complex aberration was determined using the CAB classification system . In brief, this enables an estimation of mean complex aberration size based on the minimum number of chromosomes and breaks required to form each rearrangement.

Overall, we found no evidence for an increase in complexity with increasing BrdU concentration such that the size range of complexes observed conformed to previous experimental and theoretical predictions of α-particle-induced complex aberrations in PBL . These data therefore support the prediction that aberration complexity, visible by m-FISH, is dependent on the number of different chromosome territories traversed by each individual α-particle track.

To determine whether the observed increase in complex aberration frequency could be accounted for by a reduction in break-end rejoining efficiency favouring the formation of simple exchanges, aberration complexity was assessed. According to m-FISH classification, no evidence for a shift in either a) simple to complex aberrations or in complexes of a higher order or b) in the ratio of 'complete:incomplete' complex aberrations, with increasing BrdU concentration, was seen (Table 1, Fig. 1). No difference in α-particle-induced aberration type was seen by 50 hours between the varying BrdU concentrations (S:C ratios ~0.5) (Table 1). Clearly these results are relevant only within the resolution limits of m-FISH visualisation (~11MB of DNA) and additional complexity at the molecular level cannot be excluded . Chromatid break frequency in these cells was above expected 1st division background levels (40 μM >0.6%) and could be a consequence of a BrdU-mediated effect of this kind (Table 1). Overall though, we found no evidence that the presence of BrdU decreases break-end rejoining or influences chromosome aberration complexity for α-particle irradiated PBL at this timepoint.

Exposure to ionising radiation typically results in delayed cell cycle S-phase entry. The length of cell cycle arrest is dependent on the extent and complexity of damage induced and consequently on radiation quality and dose . For cells exposed to high-LET α-particles, the appearance of 1st division PBL has been reported in PHA-

stimulated populations up to ~8 days after irradiation . In addition, only 35% of PHA-stimulated PBL exposed to high-LET iron particles were observed to be in 1st division after 72 hours of culture . In this study, we found no evidence for an alteration in population cycling time in either α-particle or sham-irradiated PBL after 50 hours in culture (Table 2). However MI values, indicative of the relative proportion of PBL reaching 1st division were seen to decline for sham-irradiated PBL with increasing concentrations of BrdU (Table 2). A similar trend was observed after exposure to high-LET α-particles, although this was not statistically significant (p=0.330). It is likely that this latter observation is a consequence of α-particle-induced cell cycle delay masking any BrdU perturbation. What these data suggest is that the presence of BrdU in the culture media will alter the rate of advancement through the cell cycle, in keeping with results obtained elsewhere . In particular, a dose-dependent association between BrdU concentration (including 10 – 40 μM range) and cell cycle progression in unirradiated cells has been demonstrated . Thus, it is likely the increased frequency of both damaged cells and complex aberrations in PBL exposed to high-LET α-particles and cultured with 40 μM compared to 0 or 10 μM BrdU, is indicative of altered cell progression producing a different fraction of the irradiated PBL population for cytogenetic sampling.

In conclusion, the parameters tested in this study are consistent with findings from previous work using m-FISH to assess chromosome aberrations after exposure to high-LET α-particle radiation both *in vitro* and *in vivo* . We show here that the presence of BrdU does not modify the size, complexity or type of high-LET α-particle induced chromosomal exchange observed by m-FISH in the 1st division PBL over a range of concentrations (0-40 μM).

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Table 1

Chromosome exchanges induced in α-particle irradiated PBL cultured in varying concentrations of BrdU

Concentration of BrdU (μM)	Total cells	Cells Damaged[a] (%)	Number of exchanges (frequency)		Total exchange frequency (%)	S:C Ratio	Number of breaks (frequency)	
			Simple (S)	Complex (C)			Chromosome	Chromatid
Sham-irradiated								
0	119	4.2	1 (0.008)	1 (0.008)	1.7	-	3 (0.025)	0 (0.000)
10	105	0.0	0 (0.000)	0 (0.000)	0.0	-	0 (0.000)	0 (0.000)
40	179	2.2	3 (0.017)	0 (0.000)	1.7	-	1 (0.006)	1 (0.006)
0.5 Gy α-particles								
0	204	15.2	14 (0.069)	25 (0.123)	19.1	0.56 ± 0.19	4 (0.020)	0 (0.000)
10	248	13.3	11 (0.044)	27 (0.109)	15.3	0.41 ± 0.15	9 (0.036)	2 (0.009)
40	145	23.4	13 (0.090)	28 (0.193)	28.3	0.46 ± 0.16	3 (0.021)	5 (0.034)

[a] Values shown include cells with chromosomal exchanges, chromosome breaks and chromatid breaks.

Table 2

Cell cycle status of α-particle irradiated cells cultured in varying concentrations of BrdU

Concentration of BrdU (μM)	Mitotic Index[a]	Harlequin analysis (%)		
		1st div	2nd div	3rd div
Sham-irradiated				
0[b]	1.77 ± 0.30	-	-	-
10	1.37 ± 0.22	96.3	3.7	0.0
40	0.61 ± 0.23	93.6	6.4	0.0
0.5 Gy α-particles				
0[b]	1.40 ± 0.25	-	-	-
10	1.36 ± 0.25	93.0	7.0	0.0
40	0.99 ± 0.18	95.5	4.5	0.0

[a] Data is pooled from two of three independent experiments.

[b] Cultures without BrdU in the growth medium cannot be Harlequin stained.

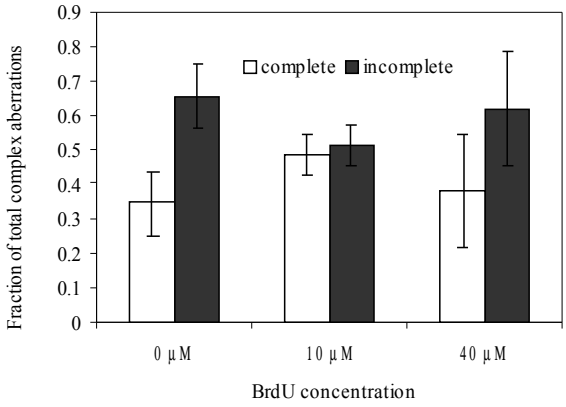
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
complete
incomplete
Fraction of total complex aberrations
0 µM
10 µM
40 µM
BrdU concentration

Fig. 1. Fraction of total complex aberrations classified as complete or incomplete in α-particle irradiated cells harvested from cultures containing 0, 10 or 40 μM BrdU. Classification is based on chromosome break-end rejoining visualised by m-FISH.

References