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# Detection of multidrug resistance and quantification of responses of human tumour cells to cytotoxic agents using flow cytometric spectral shift analysis of Hoechst 33342-DNA fluorescence

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**Summary.** We describe the application of a flow cytometric technique for assessing the radiation or drug sensitivity characteristics of human tumour cells. The technique makes use of the phenomenon that a red shift occurs in the fluorescence emission spectrum of a DNA-specific dye (Hoechst 33342) as an increasing number of dye molecules bind to nuclear DNA. Intact, viable cells undergo a time-dependent spectral shift that can be distinguished from the rapid shift observed in cells with damaged membranes by the use of multiparametric flow cytometry. The responses of various human cell lines were compared, namely, those of normal and ataxia-telangiectasia (A-T) lymphoblastoid lines, a small-cell lung carcinoma line and its (in vitro) derived multidrug-resistant variants. A close correlation was found between dye toxicity and the degree of DNA binding of Hoechst 33342 independent of cellular DNA content, with lymphoblastoid and multidrug-resistant small-cell lung cancer cells showing enhanced and restricted dye-binding rates, respectively. VP16- and radiation-induced cell kill was found to result in a quantifiable increase in the fraction of cells undergoing a rapid spectral shift and was capable of detecting the increased radiation sensitivity of A-T-derived cells. Spectral shift analysis provides a rapid method for assessing the responses of tumour cells to cytotoxic agents and for determining the general ability of cells to protect cellular DNA from a model DNA-binding agent (Hoechst 33342) that participates in the multidrug resistance phenotype.

## Introduction

The ability to identify and monitor cellular features that determine or reflect the responses of human tumor cells to cytotoxic agents is a fundamental step in the development of rational approaches to anti-cancer chemotherapy. Techniques based on flow cytometry for the evaluation of drug or radiation sensitivity in human tumour biopsies offer a means of identifying the cellular responsiveness of specified subpopulations. This paper describes the application of a flow cytometric technique to cultured human tumour cells for the determination of viability and of overall cellular capacity to limit intracellular drug availability and for the detection of changes in membrane permeability due to treatment with cytotoxic agents.

The technique relies on the measurement of the degree of violet-to-red shift in the fluorescence emission spectrum of a vital, DNA-specific bis-benzimidazole dye (Hoechst 33342) as the level of ligand bound to DNA increases [2, 14, 16]. The bis-benzimidazole dyes are AT base-pair-specific [21] and show marked fluorescence enhancement upon non-intercalative binding to DNA [9, 10, 17]. The lipophilic derivative Hoechst 33342 is used extensively as a vital nuclear stain in flow cytometry [9] but can induce DNA damage and cell death [4, 13, 15]. Current evidence suggests that the cytotoxic effects of Hoechst 33342 relate to the cellular consequences of ligand binding to the minor groove of DNA [4, 13, 15, 17, 21]. Recent studies have indicated that Hoechst 33342 mimics several classes of anti-cancer agents in the mechanisms of cellular uptake and export and participates in the multidrug resistance (MDR) phenotype in that multidrug-resistant cells show reduced Hoechst 33342-DNA fluorescence [7, 8, 11]. The MDR phenotype acts to reduce the intracellular availability of xenobiotic molecules by the operation of a pathway, dependent upon the expression of a membrane-located P-glycoprotein, for the rapid efflux of ligand molecules [6].

Dye exclusion assays for cell viability have been used extensively in various areas of cell biology [1, 3, 5] and essentially depend upon the reduced ability of a reporter molecule to penetrate intact membranes or upon the active exclusion of an agent from viable cells. The rationale behind the measurement of cell viability described in the present study is that a metabolically active cell with an intact membrane provides a partial barrier to the entry of the dye and that the appearance of nuclear fluorescence is time-dependent. The Hoechst 33342-DNA emission spectrum is initially violet biased at low ligand: phosphate

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ratios, becoming red-shifted as binding increases [2, 14, 16, 19]. We describe how spectral analysis of the fluorescence signals of Hoechst 33342-stained cells enables the identification of rapidly staining cells with damaged membranes due to drug- or radiation-induced cytotoxicity. Furthermore, a predominant dependence of Hoechst 33342 upon DNA interaction for cytotoxicity would suggest that there should be some relationship between the amount of ligand binding per unit of cellular DNA and cell kill. Thus, we compared Hoechst 33342 staining of nuclear DNA in three types of human cells that differ markedly in their sensitivity to Hoechst 33342, namely, a lymphoblastoid cell line, a small-cell lung cancer (SCLC) cell line, and two SCLC sub-lines showing MDR [6, 12].

## Materials and methods

*Cell culture and growth curves.* Five human cell lines, grown as suspension cultures, were used in the present study. NCI-H69 (kindly donated by D. N. Carney, NCI, Bethesda, Md., USA; referred to as H69) is an established SCLC cell line. The doxorubicin-resistant variants of H69 (designated NCI-H69/LX4 and NCI-H69/LX20; referred to as LX4 and LX20, respectively; kindly provided by P. R. Twentyman) were maintained under selective conditions in the presence of 0.4 and 2.0 μg doxorubicin/ml, respectively. The LX20 variant was derived from the LX4 variant by selection in increasing concentrations of doxorubicin and both sublines exhibit an MDR phenotype ([11, 12, 18]; personal communication, P. R. Twentyman). The Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines SC (normal donor) and GM 717 (ataxia-telangiectasia, or A-T, donor) were supplied by A. M. R. Taylor (Cancer Research Campaign Laboratories, University of Birmingham) and by the Human Genetic Mutant Cell Repository (Cambden, N. J.), respectively. The EBV-transformed cell lines were maintained in static suspension culture in RPMI medium supplemented with 10% foetal bovine serum, 1 mM glutamine and antibiotics and were incubated at 37° C in an atmosphere of 4%–5% $CO_2$ in air. The SCLC-derived cells grew as small aggregates of <50 cells, and large spheroid formation was prevented by repetitive pipetting (every 2–3 days) of the culture medium.

Measurements of the toxicity of Hoechst dye 33342 (CP Laboratories, Bishop's Stortford, UK) were carried out on exponentially growing cultures. Cells were inoculated into 96-well (flat-bottom) cell-culture-quality microtitre plates at densities of $1 \times 10^5$ cells/ml in growth medium (total volume, 300 μl/well) containing dilutions of Hoechst 33342. Plates were incubated for 3–6 days under normal conditions and cell densities were determined using a Coulter particle counter for protease-disaggregated cell suspensions (1 mg/ml neutral protease and 20 min incubation at 37° C).

*Flow cytometric analysis of Hoechst 33342-DNA binding.* Cells were resuspended by aspiration and diluted in medium (supplemented with 5 mM HEPES) to $2.5 \times 10^5$ cells/ml prior to Hoechst 33342 treatment and analysis using the flow cytometer previously described [11, 14, 19]. The emission spectrum of Hoechst 33342-stained DNA extends over a range of 400–600 nm. The optical analysis system at 90° to the intersection of the laser beam with the cell stream includes five dichroic mirrors in series (all from Zeiss Ltd.), with nominal 50% transmission at 390, 420, 460, 510 and 580 nm, respectively. Each dichroic mirror reflects light below the 50% transmission wavelength sequentially into a series of five photomultiplier tubes (PMT), and the last mirror in the series transmits at >580 nm into a sixth PMT. Thus, after the primary filtration due to the dichroic mirrors, PMTs 1–6 receive light in the wavelength bands of <390, 390–420, 420–460, 460–510, 510–580 and >580 nm, respectively. Additional filtration is then applied. PMT 1 was "guarded" by a UG11 black glass filter (Melles Griot, Arnhem, Holland) transmitting at <370 nm to analyse 90°-scattered light. PMTs 2, 3, 4 and 6 were "guarded" by narrow-band pass filters centred at 400, 450, 500 and 600 nm each (±5 nm; all from Melles Griot) analysing violet, low blue, blue-green and red light, respectively. PMT 5 was additionally "guarded" by a 550 nm long-pass and a 560-nm short-pass filter (both from Zeiss Ltd., giving a 555 ± 5-nm-band pass filter). Forward scatter was analysed with a solid-state detector.

The system uses a cross-cylindrical lens pair that focuses the excitation beam to a sheet measuring about $4 \times 120$ μm. Hence, peak pulse height (P), width (W, time of flight through the beam) and pulse area (A) were digitized for each cell on all seven detector channels. The data were collected in the list mode on a fast RP07 disc via a dedicated LSI 11/23 and time-sharing PDP 11/40 computers (all from Digital Equipment Corporation, DEC, Maynard, Mass., USA). The time stamp from the LSI 11/23 was also included in the data base as a quality-control check. Hence, a total of 23 integers (2 for the time stamp) were collected per cell at through-put rates between 300 and 400 cells/s.

The data were processed on a VAX 8600 computer (DEC) that extracted ten parameters for each cell from the list-mode data set. The peak pulse height, width and area from the 90°-scatter PMT, which was the master triggering detector, together with forward-scatter pulse width were used to exclude small debris and clumps. The time stamp was used to ensure that no instrumental drift had occurred during each run. Seven parameters, namely, 90°- and forward-scatter pulse width as well as pulse areas for the five fluorescence channels were then written onto the disc as a fully cross-correlated data file for subsequent display and analysis. Median fluorescence values were calculated for specified population distributions [20].

*Hoechst 33342 uptake viability assay.* Exponentially growing cultures were adjusted to $2 \times 10^5$ cells/ml and were either (a) X-irradiated at room temperature using a 250-kV machine (Pantak, Windsor, UK) operating at 15 mA with filtration of 2.32 mm of the copper half-valve thickness and a dose rate of 2.86 Gy/min or (b) exposed to VP16 (VP16-213; Vepesid; etoposide; Bristol-Myers Pharmaceuticals, Syracuse, N. Y. USA) for 30 min prior to drug removal by centrifugation and resuspension at $2 \times 10^5$ cells/ml. Following a post-treatment incubation period, cultures were adjusted to $2 \times 10^5$ cells/ml prior to exposure for 2 min (lymphoblastoid cells) or 5 min (SCLC cells) to 10 μM Hoechst 33342 at 37° C. Deoxyribonuclease treatment, when indicated, was performed prior to Hoechst 33342 staining by the incubation of cells with 1 mg/ml DNase I (Sigma) in medium containing 5 mM $MgCl_2$ for 10 min at 37° C. Samples were then analysed by flow cytometry as described above, except that fluorescence was measured at either 400- or >630-nm wavelengths. Contour plots of the fluorescence signals for cell-size particles yielded two distinct populations (see Fig. 4), the relative frequencies of which could be determined by contour gating.

## Results

### *Relative cytotoxic effects of Hoechst 33342*

The cytotoxicity of Hoechst 33342 towards several human cell lines was studied (Fig. 1). Sensitivity ranged from that of a lymphoblastoid cell line (SC; 50% growth-inhibition dose, or $ID_{50}$, <0.1 μM) to that of the doxorubicin-resistant SCLC cell lines (LX4 and LX20; $ID_{50}$, >10 μM), with the parental SCLC cell line (H69; $ID_{50}$, 0.3 μM) showing an intermediate response. Comparison of $ID_{50}$ values revealed that the sensitivity of the lymphoblastoid cell line to Hoechst 33342 was approximately 3-fold that of the H69 cell line. The H69 and doxorubicin-resistant cell lines had a similar growth rate (when cultured for 7 days in normal growth medium), with a 6-day growth period representing approximately 2.5 population doublings. The SC cell line grew more quickly, but this alone did not account for Hoechst 33342 sensitivity, since only a small increase in resistance was seen after exposure to Hoechst 33342 for 3 days (being equivalent to 2.5–3 population doublings).

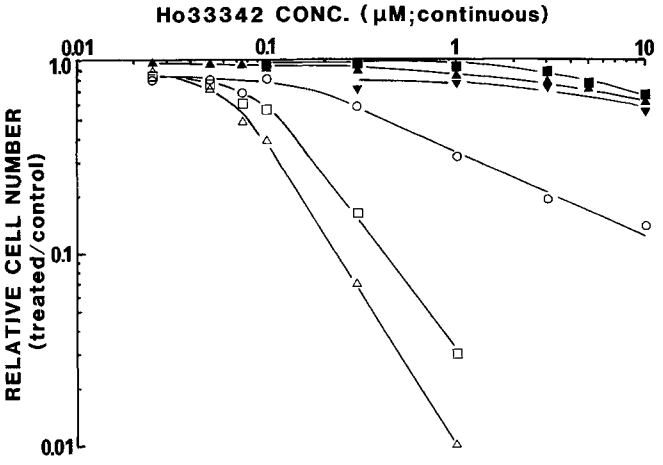


**Fig. 1.** Inhibition of culture growth by continuous exposure of human cells to Hoechst 33 342 (for 6 days, unless otherwise specified). ○, H69; ▲, LX4 (analysed immediately after removal from doxorubicin selection); ▼, LX20 (analysed immediately after removal from selection); ■, LX4 (analysed after 7 days growth in non-selective medium); △, SC; □, SC (3-day exposure to Hoechst 33 342)

*Kinetics of DNA staining by Hoechst 33 342*

We investigated the question as to whether the above pattern of Hoechst 33 342 sensitivity could be related to the relative rates at which Hoechst 33 342 could bind to nuclear DNA as monitored by flow cytometry. Analysis of the increase in fluorescence with increasing duration of exposure to Hoechst 33 342 (Fig. 2) reflects the rate at which intracellular ligand binds to nuclear DNA. As indicated by the data for fluorescence emission at 500 nm (representing the peak of the emission spectrum; Fig. 2c), it is clear that a rapid increase in fluorescence intensity occurred during the first 5 min of ligand exposure for H69 and SC cells. This rapid initial increase was followed by a plateau. For the doxorubicin-resistant SCLC cells (Fig. 2c), the initial rate of increase was much slower than that observed in the parental H69 cell line and a lower fluorescence plateau was attained. Interestingly, the LX20 cell line (with a higher level of doxorubicin resistance than LX4) showed the greatest depression of Hoechst 33 342 uptake, both in the initial rate and in the final plateau. This basic pattern changes when other regions of the emission spectrum are examined. In the violet region (Fig. 2a, b), the initial increase in fluorescence was higher and occurred more rapidly in all cell types. There was evidence of fluorescence quenching in H69 and SC cells at uptake periods of >15 min (Fig. 2a). This resulted in a small difference between the fluorescence plateau for H69 and that for doxorubicin-resistant cells, with the lowest level being observed in SC cells. In the red region (Fig. 2d, c), differences in plateau levels between H69 and doxorubicin-resistant cells were exaggerated, with the SC cells showing an intermediate response.

*Relative spectral shift in normal cells and cells expressing MDR*

Measurement of fluorescence intensity per cell (or median values for a given population) does not take into account

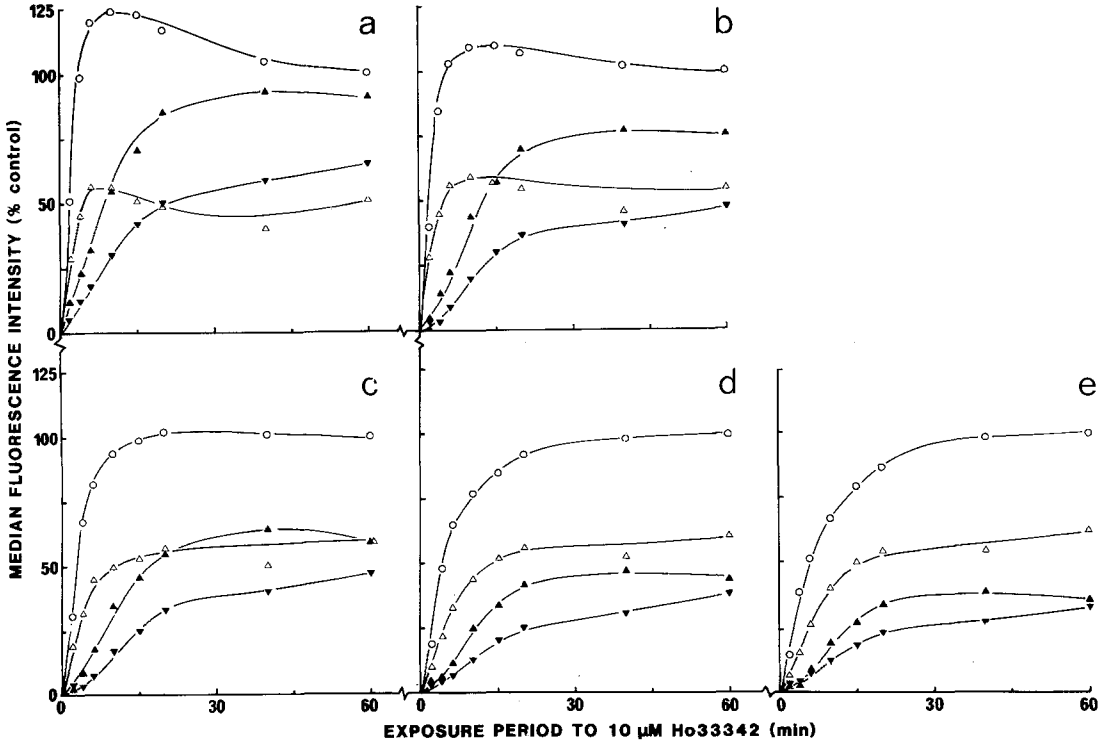


**Fig. 2a–e.** Time-dependent increase in nuclear fluorescence for cells exposed to Hoechst 33 342 (10 μM) and monitored at wavelengths of **a** 400 nm, **b** 450 nm, **c** 500 nm, **d** 550 nm, and **e** 600 nm. For each wavelenght, monitored data are expressed relative to an H69 control culture exposed to dye for 1 h. ○, H69; ▲, LX4; ▼, LX20; △, SC

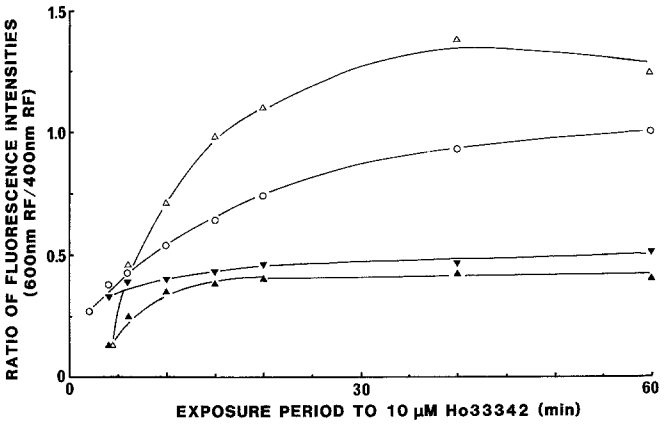


**Fig. 3.** Relative red shift in the Hoechst 33342-nuclear DNA emission spectrum during the course of Hoechst 33342 exposure (data derived from values given in Fig. 2). ○, H69; ▲, LX4; ▼, LX20; △, SC

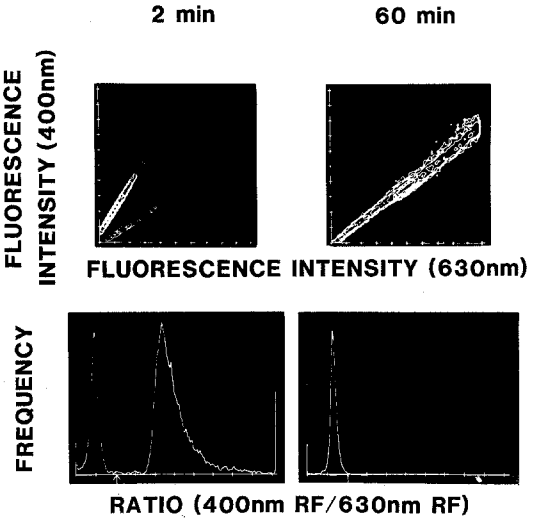


**Fig. 4.** Differential fluorescence of H69 cell subpopulations according to the period of exposure to Hoechst 33342. *Upper panels* show contour plots and the *lower panels* show the distribution of ratio values for the fluorescence signals. Two distinct populations are apparent at 2 min but not at 60 min Hoechst 33342 exposure periods. A slowly staining population (SSP) demonstrates a high (*400 nm RF/630 nm RF*) ratio, whereas a rapidly staining population (RSP) shows no significant change in ratio during the 2–60 min incubation period. The staining kinetics of the SSP are shown in Fig. 2

the differences in ligand:phosphate ratios or the variation in DNA content per cell for different cell lines. At a given wavelength the fluorescence intensity is dependent upon the quantity of ligand bound to DNA. However, the quantity of ligand that can bind to DNA is proportional to the amount of DNA per cell and to the amount of ligand that can gain access to DNA. By taking the ratio of two fluorescence intensities at different wavelengths from the same cell (e.g. 600- and 400-nm wavelengths; Fig. 3), one can follow the relative change in ligand:phosphate ratios (irrespective of cellular DNA content), as binding sequences are effectively titrated by ligand molecules during the course of Hoechst 33342 exposure. Figure 3 compares the degrees of spectral shift for the four cell types and reveals that the shift was greater for SC cells than for H69 cells, with the doxorubicin-resistant cells showing the least change. Considering the time taken to increase the 600 nm/400 nm ratio from 0.25 to 0.75, SC and H69 cells give values of 6 and 19.1 min, respectively (Fig. 3). The results imply that the SC cell line binds more Hoechst 33342 per unit DNA than does the H69 cell line. The doxorubicin-resistant cells showed significant limitations on the progressive ability of Hoechst 33342 molecules to bind to DNA during the course of ligand exposure, with 600 nm/400 nm ratio values reaching a plateau of <0.5 after 20 min of Hoechst 33342 exposure. This block to the time-dependent ability of ligand molecules to accumulate on cellular DNA is consistent with the high level of resistance to cell killing shown by the doxorubucin-resistant cell lines.

*Spectral shift as a monitor of cell viability*

In early studies (data not shown) we observed that cell cultures that carried a significant proportion (>5%) of trypan blue-positive cells, due to growth-medium starvation, showed two distinct populations during early staining (<10 min exposure to 10 μM) with Hoechst 33342. A rapidly staining population (RSP) and a slowly staining population (SSP) were characterised by low and high 400-nm/630 nm ratios, respectively. The fluorescence intensity of the RSP was stable within 2 min of dye addition. The kinetics of nuclear staining for the SSP are shown in Figs. 2 and 3. Preliminary studies showed that the intensities of staining in the RSP and SSP were dependent upon dye concentration. However, concentrations of >15 μM resulted in significant quenching of the RSP fluorescence and those of <5 μM resulted in a less distinct DNA histogram for the SSP after 1 h exposure. Thus, a dye concentration of 10 μM was selected to establish a convenient condition for discerning these two populations by spectral shift analysis. Figure 4 shows the RSP and SSP fractions (see legend for details) for H69 cells, displayed either as con-

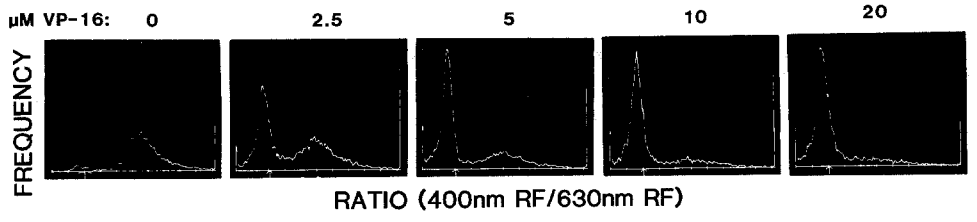


**Fig. 5.** Change in the short-term (2 min exposure to 10 μM) Hoechst 33342 staining patterns of lymphoblastoid (SC) cells as a function of VP16 treatment. Cultures were assayed 72 h after a 30-min exposure to VP16 and show a progressive loss of the SSP (see legend to Fig. 4)

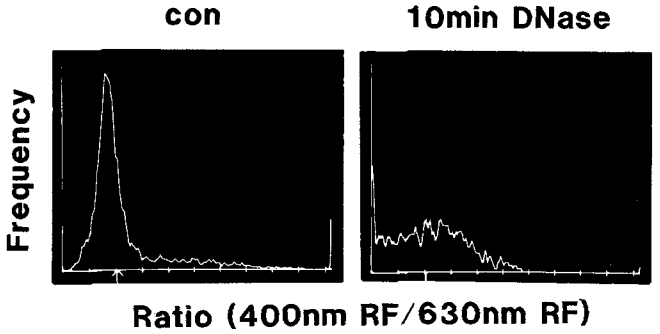


**Fig. 6.** Sensitivity of the Hoechst 33 342 nuclear fluorescence of VP16-inactivated (20 μM VP16; see Fig. 5) lymphoblastoid (SC) cells to exogenously supplied DNase I

**Table 1.** Effects of VP16 or X-irradiation on cell viability as determined by Ho33 342 uptake

| Cell line | Treatment (units) | Dose reducing SSP fraction to 50% of control[a] for various post-treatment incubation periods | | | |
|---|---|---|---|---|---|
| | | 24 h | 48 h | 72 h | 96 h |
| NCI-H69 | VP16 (μM)[b] | >20[c] | >20 | 6.2 | 6.2 |
| SC | VP16 (μM)[b] | 5 | 3.8 | 2 | 2 |
| SC | X-rays (Gy) | 2.8 | 1.8 | 1.8 | ND |
| GM717 | X-rays (Gy) | 1.9 | 0.8 | 0.7 | ND |

[a] Dose reducing the ratio of {[SSP/RSP+SSP]$_{treated}$/[SSP/RSP+SSP]$_{control}$} to 0.5; see Materials and methods and the legend to Fig. 4 for details
[b] 30 min exposure
[c] Data shown for representative experiments
ND, Not determined

tour plots or as ratio distributions of 400- and >630-nm fluorescence signals. The SSP had characteristics identical to those of the cells monitored in the experiments described in Fig. 2, whereas the RSP population showed no time-dependent shift in the Hoechst 33 342 fluorescence emission spectrum and was presumed to be both in equilibrium with the dye and equivalent to a trypan blue-positive fraction.

The proportion of cells in the RSP fraction was found to increase following treatment with a cytotoxic agent such as VP16, and Fig. 5 shows typical results for the SC cell line. The increase in the RSP fraction was both time- and dose-dependent but was always greater than that determined in the non-viable fraction by trypan blue exclusion. For example, 24 h after treatment with 0, 2.5, 5, 10 or 20 μM VP16, the percentages of the total cells showing trypan blue-positive staining amounted to 8.9%, 7.2%, 11.4%, 17.7% and 19.6%, respectively, whereas those in the RSP fraction were <1%, 31%, 50%, 57% and 60%, respectively. The nuclear fluorescence of cells appearing in the RSP fraction was sensitive to exogenously supplied DNase I (Fig. 6), whereas cells in the SSP fraction were unaffected by the presence of the enzyme (data not shown). DNase I sensitivity is consistent with cells in the RSP fraction's having damaged membranes. The results suggest that the Hoechst 33 342 uptake viability assay is relatively more sensitive than trypan blue exclusion at the low dose levels of VP16. The loss of cells from the SSP fraction was time-dependent (Table 1), with an incubation period of 48–72 h being required to establish a stable dose-response curve. Incubation periods of >96 h resulted in treated cultures containing significant levels of cell debris, suggesting the onset of cell lysis.

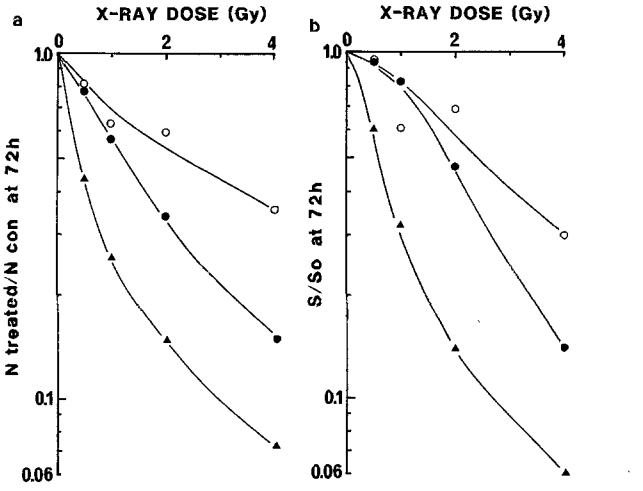


**Fig. 7 a, b.** Relative sensitivity of human cells to X-irradiation. **a** Data represent the cell density of treated cultures relative to the untreated control at 72 h following irradiation. **b** Data represent the proportion of total cells remaining in the SSP at 72 h following irradiation (i.e. {[SSP/RSP+SSP]$_{treated}$/[SSP/RSP+SSP]$_{control}$}; see legend to Fig. 4 for details). ○, H69; ●, SC (normal lymphoblastoid); ▲, GM717 (A-T lymphoblastoid)

To determine whether the Hoechst 33 342 uptake viability assay could detect differences between cells of different sensitivities to a given agent, we compared the effects of X-irradiation on an SCLC cell line (H69) with the responses of lymphoblastoid cell lines from a normal or A-T radiation-sensitive donor. Figure 7 a shows the effects of radiation on the relative growth rate and indicates that radiation sensitivity increases in the order H69 <SC <GM717. The Hoechst 33 342 uptake viability assay (Fig. 7 b) shows a similar pattern. Thus, within the growth-arrested populations of cells monitored by cell counting (Fig. 7 a), an increasing proportion fall into the RSP fraction as the radiation dose increases. A post-irradiation incubation period of up to 48 h (Table 1) was required to establish the maximal distinction between the normal (SC) and the A-T (GM717) radiation responses.

## Discussion

In the present study we made use of the observations that there is a red shift in the fluorescence emission spectrum of Hoechst 33 342 bound to nuclear DNA as the ligand:phosphate ratio increases and co-operative binding events become more frequent [2, 14, 16, 19]. During the time-dependent uptake of the ligand the potential nuclear binding sites are effectively titrated, with the initially violet-biased emission spectrum undergoing a shift in emission maximum to longer wavelengths. Consequently,

monitoring the time-dependent shift in the emission spectrum provides information on the rate of ligand binding, whereby the absolute DNA content per cell is no longer a factor. Fluorescence microscopy of the stained cells (data not shown) reveals intense nuclear fluorescence, and it is unlikely that the spectral shift, within the present experimental limits, is affected by cytoplasmic accumulation of dye molecules. Indeed, both cells rendered permeable and isolated chromatin also demonstrate the shift phenomenon [14, 16].

The above approach enabled us to identify an elevated capacity of lymphoblastoid cell nuclei as compared with nuclei of an SCLC cell line (H69) to bind Hoechst 33342 molecules. On the other hand, the operation of the MDR pathway [7, 8, 11] drastically reduces the opportunity for nuclear binding of Hoechst 33342 in the variant SCLC cell lines. The patterns for nuclear DNA protection against Hoechst 33342 binding correlate with the resistance to the cytotoxic effects of the ligand. These results are consistent with the general observation that Hoechst 33342-DNA binding is a major factor in cell killing by the ligand. We assume that the cell types studied do not differ in the overall frequency of potential ligand-binding sites on DNA but may differ in a number of cellular features that can affect the frequency of bound molecules. Further studies are required to determine whether the restriction on the level of ligand interaction with DNA of SCLC cells is due to factors that govern intracellular ligand concentration alone or to other features such as the discrete accessibility of DNA sequences within chromatin. The findings may explain the elevated sensitivity of lymphoblastoid cells as compared with other cell types to DNA-binding drugs such as mAMSA and doxorubicin. Accordingly, lymphoblastoid cells may present a more open chromatin structure or exert less stringent control over the nuclear exclusion of xenobiotic molecules.

An extension of the spectral shift technique has enabled the development of an assay for cytotoxic drug- or radiation-induced cell inactivation. At present, the relationship between changes in membrane permeability detected by Hoechst 33342 staining and cell viability is empirical but appears to provide a more sensitive monitor of cellular integrity than that offered by trypan blue staining. A recent report [5] has described the detection of a concentration-independent spectral shift of Hoechst 33342 fluorescence to longer wavelengths in cytotoxic drug-treated haemopoietic cells. Demonstration of this shift required long-term (2-h) incubation with the ligand and may reflect structural changes in the chromatin in dead cells [5]. The Hoechst 33342 uptake viability assay (proposed acronym, HUVA) described herein offers the advantage that the rate of spectral shift in the viable (SSP) population can provide independent information on the MDR status of a cell population or the ability of a given cell to limit nuclear binding of a model DNA-binding drug, namely, Hoechst 33342.

We envisage that this flow cytometric technique could be used to identify intact viable cells in biopsy samples and to follow their responses to cytotoxic agents given either in vivo or in short-term in vitro culture. An advantage would be that biopsies containing relatively low viable fractions of cells could be assessed. The non-destructive nature of the technique should enable simultaneous measurements of other biological characteristics such as the expression of a specific enzyme or marker protein, including the MDR-related membrane P-glycoprotein [6], by the use of dual-label flow cytometry or cell-sorting techniques.

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