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cis-Diamminedichloroplatinum(II)-induced Sister Chromatid Exchange: An Indicator of Sensitivity and Heterogeneity in Primary Human Tumor Cell Cultures[1]

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ABSTRACT

The effect of *cis*-diamminedichloroplatinum(II) (cPt) on sister chromatid exchange (SCE) induction was determined in 13 human primary tumor cell cultures. Primary cultures were derived from surgical specimens of solid tumors composed of a variety of histologies. Three to 16 days after biopsy, depending on the growth rate, cultures were treated with graded concentrations of cPt for 1 h and the SCE assay was performed. SCE dose-response curves (SCEs induced per chromosome *versus* cPt concentration) showed a wide range in cPt sensitivities that was not dependent on histology. SCE frequency histograms showed that several of the primary cultures contained both cPt-sensitive and -resistant cells. For six of the cultures, the SCEs induced per chromosome at 15 μM cPt were plotted *versus* the IC_{90} determined from a survival assay. A line fit to those points yielded a correlation coefficient of −0.74. These results show a relationship between the activity of cPt in the SCE assay and in the survival assay, which suggests that SCE analysis may be useful for predicting cPt sensitivity. In addition, characterization of cellular heterogeneity in cPt sensitivity using the SCE assay may provide additional information useful in the prediction of tumor response to treatment.

INTRODUCTION

Although the precise molecular mechanism responsible for SCE[3] formation remains undefined, the induction of SCEs is generally thought to reflect some type of DNA damage (1). Qualitative correlations between SCE induction and the induction of mutations (2) and neoplastic transformation (3) have been established, resulting in the routine use of the SCE assay in assessment of mutagenic and carcinogenic potential of various compounds. As it is for mutagens/carcinogens, the principal target of many antineoplastic drugs is thought to be nuclear DNA. Thus, the induction of SCEs in cells treated with many cancer chemotherapeutic agents may also be indicative of cellular drug sensitivity. In both sensitive and resistant rat 9L brain tumor cells grown *in vitro*, the induction of SCEs by BCNU and other nitrosoureas has been correlated with cell kill; a linear increase in SCEs is detected at doses corresponding to the shoulder region of the cell survival curves (4). Similar correlations hold for other rodent cell lines and established human brain tumor cell lines and with other drugs (5, 6). In addition, agents that modify drug-induced cell kill (α-difluoromethylornithine, X-rays, dimethyl sulfoxide, and *N*-methylformamide) also modify the levels of drug-induced SCEs (7–10); in all cases the SCE assay provides the same relative information as does the conventional cell survival assay. Finally, it has been shown recently that the *in vitro* SCE assay predicts the *in vivo* response of a murine hepatocarcinoma and its pulmonary metastases to three commonly used chemotherapeutic agents, cPt, BCNU, and melphalan (11).

Because the SCE assay is based on the analysis of individual cells, SCE induction has the additional advantage of allowing the quantitation of the proportion of cell types in a mixture containing both drug-sensitive and -resistant cells. This is accomplished by expressing SCE data as a frequency histogram (the number of SCEs per metaphase or chromosome *versus* the number of cells), thus identifying distinct regions of drug-sensitive and -resistant populations; the approximate percentages of sensitive and resistant cells in each mixture can be estimated by measuring the relative areas of the two regions (12). Thus, the SCE assay has the potential for identifying heterogeneity in drug response within a tumor.

Although results obtained from experimental tumor systems suggest that the SCE assay may be useful in the *in vitro* prediction of human tumor chemosensitivity, in order to further investigate the potential clinical applicability of the SCE assay it was necessary to extend these types of studies to primary cultures of human tumors. A new tumor cell primary culture system has recently been described that makes use of CAM (LifeTrac, Irvine, CA) for cell attachment and an enriched culture medium (13). This system was used to test the sensitivity of primary human cell cultures to antineoplastic agents and for studying drug-induced SCEs. This report describes cPt-induced SCEs in primary cell cultures derived from human tumor specimens and includes a direct comparison of cPt sensitivity predicted by both SCE and *in vitro* cell survival.

MATERIALS AND METHODS

Cell Preparation and Culture. Biopsy and surgical specimens from solid human tumors were obtained from the Department of Pathology of the University of Texas M. D. Anderson Hospital and Tumor Institute at Houston. Specimens were minced with scalpels and disaggregated into single cells by enzymatic procedures (13). Cells were inoculated onto culture surfaces coated with CAM (13). Growth medium consisted of Ham's F-12 with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 10% swine serum, penicillin-streptomycin supplemented with transferrin, hydrocortisone, epidermal growth factor, and insulin as described (13). The initial attachment medium consisted of the above medium plus 0.6% methylcellulose. After 24 h of incubation at 37°C in a 95% air, 5% CO_2 atmosphere, the attachment medium was removed, the adherent cells washed with phosphate-buffered saline, and growth medium added.

Survival Assay. The cell survival assay using the adhesive tumor cell culture system has been described recently (13). This survival assay is based on measurements of cell density in treated and untreated cultures after 14 days of growth and the results correlate well with cell killing (13). cPt was added 24 h after inoculation and removed 4 days later. After an additional 7 days of incubation, the cultures were fixed and

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[3] The abbreviations used are: SCE, sister chromatid exchange; cPt, cis-diamminedichloroplatinum(II); CAM, cell-adhesive matrix; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea.

stained, and the survival at each dose was determined by quantitating total staining density by digital image analysis (13). Drug sensitivity was expressed as the dose of cPt required to kill 90% of cells (IC_{90}).

SCE Assay. Cells (5–7.5 × 10^5) were inoculated as described above onto 100-mm CAM-coated tissue culture dishes. After being fed 24 h later, cells were incubated until they covered approximately 20–30% of the culture dish surface. Cultures were treated with graded concentrations of cPt for 1 h and rinsed with phosphate-buffered saline, and then 10 ml of fresh growth medium containing 10 μM bromodeoxyuridine was added. Culture dishes were wrapped in aluminum foil and the cells allowed to replicate for 2 cycles (48–96 h) before harvesting. Cultures were treated with Colcemid (0.04 μg/ml) 2 h before being harvested by trypsinization (0.05% trypsin containing 1 mM EDTA). The cells were pelleted by centrifugation, resuspended in 0.075 M KCl for 15 min, and then fixed and washed in freshly prepared methanol:acetic acid (3:1). Sister chromatids were differentially stained using the method of Perry and Wolff (14).

RESULTS

The primary cultures used in this study were initiated from specimens obtained from a variety of tumor types (Table 1). The average time between tumor biopsy and cPt treatment was 6.5 days, with a range of 3–16 days. The time between treatment and collection was based on the growth rate of the cultures. Most cultures were collected 72 h after treatment; slower growing cultures (for example, 3422) were allowed 96 h and faster growing cultures (3510) were collected in 48 h. Background SCE levels in untreated cultures varied from 0.13–0.26 SCEs/chromosome. In this limited sample number, no correlation was detected between background SCEs and tumor histology and no difference in background SCEs was found between samples obtained from primary tumors and those obtained from metastases.

The SCE dose-response curves obtained after cPt treatment of these primary cultures are shown in Fig. 1. In order to compare the relative cPt sensitivities of the primary cultures with respect to SCE induction, the corresponding background levels were subtracted from the counts for each treatment group and the data expressed as number of SCEs induced per chromosome. As shown, the primary cultures exhibited a wide range in the levels of cPt-induced SCEs, with the most sensitive being 3498 and 3500 and the least sensitive being 3377. cPt sensitivity did not appear to be dependent on histological type, although this is uncertain because of our small sample size.

Of the tumor cultures listed in Table 1, the cPt sensitivity was determined in six cultures (using the adhesive tumor cell survival assay) for which SCE data were obtained at 15 μM. The number of SCEs induced per chromosome at 15 μM cPt

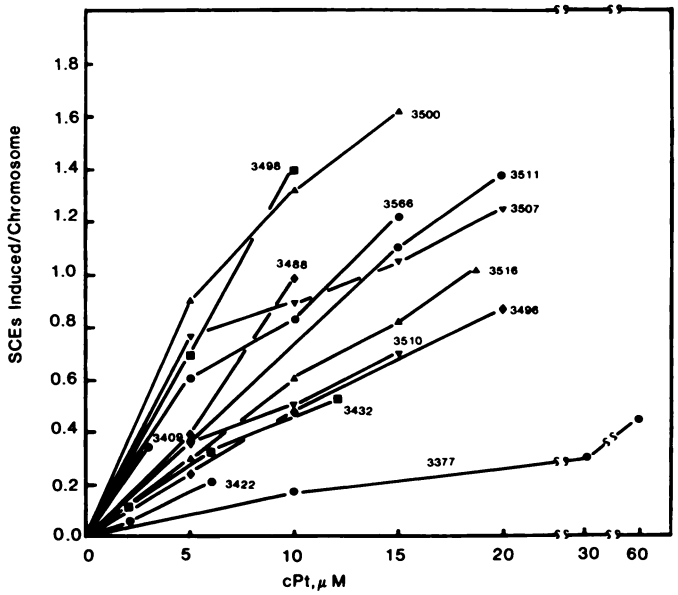

Fig. 1. SCE dose-response curves for 13 human tumor primary cultures. Cultures were treated for 1 h with graded concentrations of cPt and the SCE assay was performed. Background levels of SCEs have been subtracted. *Points*, mean values of 10–40 metaphase cells. For clarity, error bars have been omitted; the standard errors ranged from 4–15% of the mean.

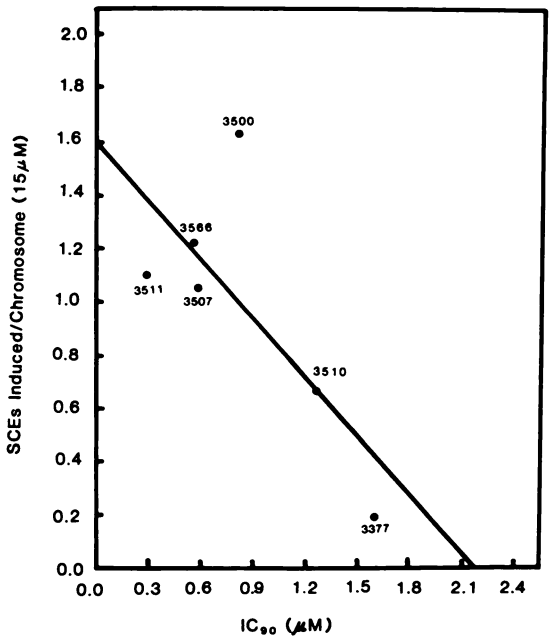

Fig. 2. Correlation between SCEs/chromosome induced by 15 μM cPt and the IC_{90} for cPt determined from the survival assay. The line was drawn according to least-squares analysis. The correlation coefficient is −0.74.

Table 1 *Histology, days in culture, and background SCE levels of 13 human tumor primary cultures*

Access number	Histology[a]	Days between biopsy and assay	Background SCEs/chromosome[b]
3377	Melanoma, m	5	0.13 ± 0.016
3409	Melanoma, m	7	0.16 ± 0.016
3422	Sarcoma, m	16	0.16 ± 0.018
3432	Germ cell, p	10	0.17 ± 0.011
3488	Bronchogenic carcinoma, p	4	0.14 ± 0.013
3496	Colon carcinoma, m	10	0.14 ± 0.016
3498	Unclassified sarcoma, m	6	0.14 ± 0.011
3500	Melanoma, m	4	0.18 ± 0.011
3507	Lung adenocarcinoma, p	7	0.21 ± 0.016
3510	Rhabdomyosarcoma, m	3	0.26 ± 0.040
3511	Lung squamous cell carcinoma, p	5	0.20 ± 0.018
3516	Ovarian carcinoma, p	3	0.18 ± 0.018
3566	Neurofibrosarcoma, m	4	0.20 ± 0.011

[a] Histological classifications were confirmed by a pathologist. Biopsies were obtained from either a metastasis, m, or primary tumor, p.
[b] Values represent the mean ± SE of 20 metaphase cells.

was plotted against the cPt IC_{90} as determined by the survival assay for each primary culture (Fig. 2). As a measure of cPt sensitivity using the SCE assay, SCE induction at a cPt concentration of 15 μM was chosen because it provided the greatest range of sensitivities among the samples. Linear least-squares analysis of the data shown in Fig. 2 revealed a correlation coefficient of −0.74. Thus, for these two assays, with the data expressed as an average value for the cells analyzed, a reasonable correlation was obtained.

To determine cPt response on an individual cell basis, results were expressed as SCE frequency histograms. Histograms were generated from representative cultures shown in Fig. 1, for which SCE data were obtained at 5 μM cPt (Fig. 3). In contrast to the SCE dose-response data, which used 15 μM cPt as the

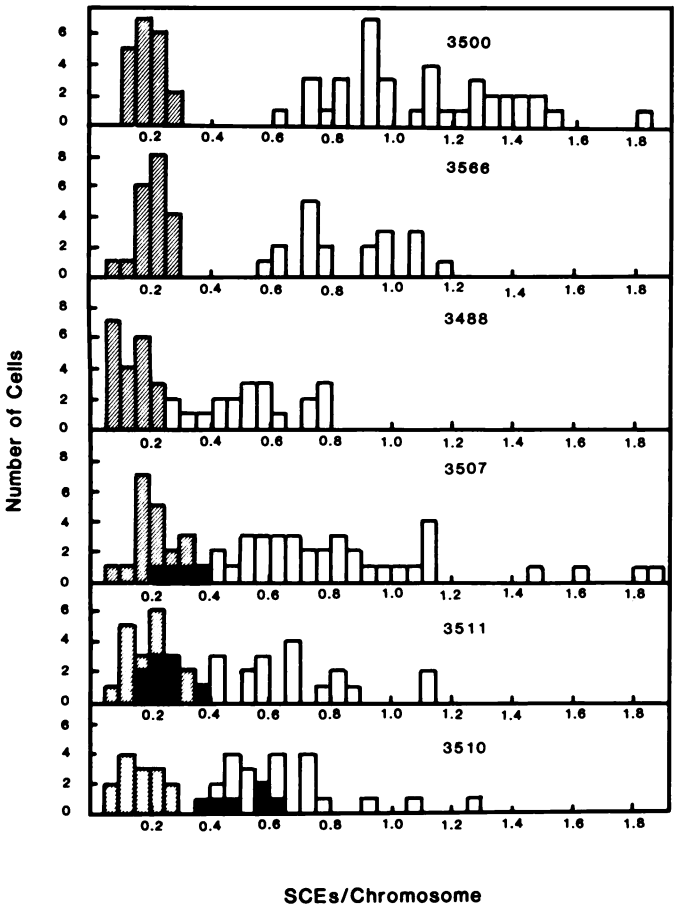

Fig. 3. SCE frequency histograms obtained by plotting the number of cells *versus* SCEs/chromosome. *Hatched areas*, untreated cells; *open areas*, cells treated with 5 μM cPt; *blackened areas*, regions occupied by both untreated and treated cells (*overlap*).

end point for comparison to the cell survival assay, 5 μM cPt was chosen for histogram analysis in order to minimize the potential growth inhibitory actions of cPt on sensitive cells. A cPt-induced slowing of the growth rate would result in a lower proportion of the more sensitive cells completing two cell divisions, thereby producing an overrepresentation of resistant cells as second-division metaphases. Each histogram contains data from both treated (5 μM cPt) and untreated cultures, thus allowing for a direct comparison between cells that contain only background SCEs and cells that contain both background and cPt-induced SCEs. As shown in Fig. 3, there is a clear distinction between the control and treated populations of cultures 3566 and 3500, demonstrating that all cells scored were sensitive to cPt, albeit to various degrees. However, treated cultures analyzed from cultures 3507, 3510, and 3511 had significant areas of overlap with their untreated controls. This suggests that these three cultures contained both cPt-sensitive and -resistant cells. With respect to culture 3488, there is no clear separation between treated and untreated cells, yet no overlap was detected in the analysis of 20 cells. In previous studies using established cell lines at least 50 metaphase cells were scored when using the SCE assay to evaluate heterogeneity in drug response (11, 12). In these initial studies using human primary cultures we were able to obtain only 20–40 scorable metaphase cells per culture. However, the histograms for cultures 3507, 3510, and 3511 still clearly demonstrate the existence of cPt-sensitive and -resistant populations. The relatively small number of cells analyzed, however, does not preclude the possibility that a minor population of cPt-resistant cells also existed in cultures 3500, 3566, and 3488.

DISCUSSION

The availability of a method for prospectively determining the chemosensitivity of tumors would provide obvious benefits in the clinical management of cancer. Investigations into the development of predictive assays have focused primarily on clonogenic cell survival (15) and, more recently, on survival based on cell growth (13). However, results obtained from established cell lines suggested that SCE analysis may also have potential applicability as a predictive assay of tumor drug sensitivity. The data presented in this report, in which the sensitivity to cPt in the SCE and survival assays correlated for human tumor cells in primary culture, lend further support to the potential predictive usefulness of the SCE assay. Although the cPt SCE dose responses of only 13 primary cultures are shown in Fig. 1, our initial studies using cPt concentrations of less than 3 μM (a concentration later shown to be too small to elicit significant SCE induction in human primary cultures) resulted in SCE data for an additional 12 primary cultures (results not shown). Thus, to date, the SCE assay after cPt treatment has been attempted on 38 primary cultures, and scorable metaphases were obtained on 25, making the success rate approximately 66%. We have continued to modify and improve the assay; thus, we expect that the success rate will continue to increase as our experience with primary cultures increases.

The data obtained from survival assays are based on the average response of an entire treated population, which, for a homogeneous cell population, is an accurate reflection of drug sensitivity. However, human tumors often contain cell subpopulations that have different drug sensitivities. Heterogeneity confounds the prediction of tumor chemosensitivity and is a factor that is not easily addressed by assays based on the average cellular response. The presence of a minor subpopulation of resistant cells would not be detected by the survival assay, which would be dominated by sensitive cells, since it uses a concentration that reduced the survival to 10% (IC_{90}) as a comparative end point. This minor subpopulation would also not be detectable by examining SCE dose-response curves. However, since the SCE assay involves the analysis of individual cells, expression of SCE data as frequency histograms provides information regarding the various drug responses existing among tumor cell subpopulations. This is best exemplified in the histograms generated for cultures 3507, 3510, and 3511, which suggest that perhaps as many as 20% of the cells analyzed are resistant to cPt. Provided the induction of SCEs *in vitro* truely represent the *in vivo* cell drug sensitivity, this type of information would be of value in the design of individual treatment protocols.

Even though the correlation between the SCE assay and the survival assay is good, a potentially significant technical factor likely to decrease the correlation between the two assays is the cPt treatment time: 96 h for the survival assay and only 1 h for the SCE assay. cPt uptake and cell cycle effects vary considerably between these two treatment protocols. Future studies are being designed using a 1-h cPt treatment for both assays. However, since only the relative ranking of cPt sensitivity is being compared and a fairly good correlation was obtained (Fig. 2), it would appear that the difference in treatment protocols does not significantly influence the sensitivity ranking for the majority of cultures.

The application of SCE analysis as a predictive test for tumor

chemosensitivity offers at least two distinct advantages: evaluation of heterogeneity and the requirement for only two cell divisions *in vitro* as compared with five or six for survival assays. The use of the SCE assay obviously would be limited to specific drugs for which correlations between cell survival and SCE induction have been established in an experimental setting. To date this has been accomplished for cPt, BCNU, and melphalan (4, 5, 11), agents commonly used in cancer treatment. The data presented here, demonstrating the feasibility of performing the SCE assay on primary cultures and the ability of the SCE analysis to detect heterogeneity in cPt response, indicate that further investigations on the clinical use of the SCE assay as a predictive method are warranted. The most appropriate potential application of SCE analysis may be as a complimentary method to the survival assay for the evaluation of heterogeneity in drug response in cultures predicted to be sensitive to a specific chemotherapeutic agent.

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