ABSTRACT

Clinical studies have suggested a close correlation between cis-diamminedichloroplatinum(II) (cisplatin) and radiation resistance. To determine whether this cross-resistance is due to an inherent cellular resistance to both agents, ten early passage human tumor cell lines were examined for their radiation and cisplatin sensitivity in vitro. Previous studies have suggested that these early passage tumor cell lines retain many of their in vivo characteristics and are therefore good models for tumor cells in vivo. Radioreistance was strongly associated with cisplatin resistance in these cell lines. Four of the cell lines examined were radioreistant, having D50 > 2.0 Gy. These four lines were also resistant to cisplatin, with the dose reducing survival to 10% > 1.29 µM. The remaining six cell lines had D50 ranging from 1.07 to 1.57 Gy of X-ray and doses reducing survival to 10% of less than 0.83 µM cisplatin. Because early passage human tumor cell lines were used, resistance or sensitivity to radiation and cisplatin most likely developed in vivo and was not due to selection in vitro. These results indicate that cross-resistance between cisplatin and radiation in vivo is probably due primarily to an inherent cellular resistance to these agents and not necessarily to the tumor microenvironment in situ.

INTRODUCTION

cis-Diamminedichloroplatinum(II) (cisplatin) and ionizing radiation are two antineoplastic agents which have had some effectiveness in the treatment of malignancies when used either alone or in combination with each other. Clinical studies have suggested that cross-resistance between radiation and cisplatin is a common occurrence in human tumors. Both Ervin et al. (1) and Enssley et al. (2) have reported a close correlation between resistance of head and neck tumors to cisplatin therapy and resistance to radiation therapy. Cross-resistance of human tumors to cisplatin and radiation could be due to changes in the tumor microenvironment in situ. For example, reductions in blood flow to the tumor could reduce the uptake of cisplatin into the tumor leading to cisplatin resistance. Similarly, reduced blood flow would also reduce intratumor oxygen levels, leading to hypoxia, which is postulated to increase radiation and chemoresistance. Alternatively, the presence of inherently drug/radiation-resistant cells within the tumor might result in a cross-resistance phenotype. A number of studies have suggested that the presence of inherently radioresistant or drug-resistant cells within a tumor might underlie cancer therapy failure (3-5). Cross-resistance in vivo might be due to inherent cellular factors which make tumor cells resistant to both radiation and cisplatin.

In vitro studies on the relationship between cisplatin and radiation resistance have had contradictory conclusions. Louie et al. (6) reported a close correlation between radiation and cisplatin resistance in a human ovarian tumor cell line, selected for resistance to cisplatin by continuous exposure to the drug. In contrast, Wallner and Li (7), studying radiation resistance in a Chinese hamster fibroblast cell line also selected for resistance to cisplatin by continuous growth in progressive concentrations of the drug, found no relation between cisplatin and radiation resistance.

Both Louie et al. (6) and Wallner and Li (7) studied cell lines selected for resistance to cisplatin by growing cells in progressively higher concentrations of cisplatin in vitro. To examine the relationship between radiation and cisplatin resistance as it develops in vivo in human tumor cells, we have examined radiation and cisplatin resistance to 10 early passage human tumor cell lines derived from a variety of human tumor biopsies. Repeated analysis of radiation and cisplatin sensitivity requires clonal cell lines. While changes in tumor phenotype have been reported in long-term in vitro cultures (8), there is much evidence that these early passage cultures of tumor cell lines do maintain their in vivo features (9, 10).

MATERIALS AND METHODS

Ten human tumor cell lines (6 squamous cell carcinomas, 2 soft tissue sarcomas, and 2 adenocarcinomas) and one nontransformed normal human fibroblast cell line (AG1522) were examined. A summary of their origins is found in Table 1. Tumor cell lines were established as described by Rheinwald and Beckett (9, 10). They were maintained in medium consisting of 72.5% Dulbecco’s modification of Eagle’s medium, 22.5% Ham’s Nutrient Mixture F-12, 5% fetal calf serum, 20 ng/ml of epidermal growth factor (added the third day after plating), 5 µg/ml of insulin, 5 µg/ml of transferrin, 2 x 10⁻⁹ M 3, 3’,5'-triiodo-1-thyronine, 10⁻⁶ M cholera toxin, 1.8 x 10⁻⁵ M adenosine, 0.4 µg/ml of hydrocortisone, 100 units/ml of penicillin, and 100 µg/ml of streptomycin. The nonmalignant cells (AG1522) were maintained in minimal essential medium supplemented with 10% fetal calf serum, 100 units/ml of penicillin, and 100 µg/ml of streptomycin.

Human tumor cells (mostly at passages 2 to 15) were maintained in medium at 37°C in a humidified atmosphere of 5% CO₂ in air. Cells were trypsinized with 0.05% trypsin from stock cultures, and between 500 and 40,000 cells were plated in 10-cm-diameter dishes and allowed to enter exponential growth. Irradiation or treatment with cisplatin was carried out 18 h later.

Cells were irradiated with a General Electric Maximair X-ray generator at 200 kVp and 15 mA yielding a dose rate of 0.8 Gy/min. Immediately after irradiation, the cultures were returned to the incubator. After 18 to 24 days, the cells were fixed and stained with crystal violet. Only colonies of more than 50 cells were scored as survivors. All experiments were repeated 2 to 4 times. The standard errors were always less than 10%.

The radiation survival curve parameters determined were the D₀, which is the inverse of the slope of the survival curve, and the extrapolation number (n), which is the back extrapolation of the slope to the ordinate. These parameters were determined by a least-squares regression analysis of all the data points. The radiosensitivity is defined by the D₀, while n is a reflection of the cell’s ability to accumulate sublethal damage (11, 12).

Cisplatin was prepared freshly just before use. All exposures were...
The X-ray survival curves for the 10 human tumor cell lines and AG1522 normal fibroblasts are shown in Fig. 1. From the survival curve data, $D_0$ and $n$ were determined as described in “Materials and Methods.” The extrapolation numbers for all the data points. The survival curves for cisplatin-treated cells are shown in Fig. 2. From the survival curves, $IC_{50}$ was calculated and are shown in Table 1. $IC_{50}$ ranged from 0.66 $\mu M$ in the OVC-1 cell line to 1.9 $\mu M$ in the OVC-2 cell line. The four radioresistant cell lines, JSQ3, SQ20B, SCC-12B2, and OVC-2, were also cisplatin resistant, having $IC_{50}$s of 1.68, 1.29, 1.43, and 1.90 $\mu M$ cisplatin, respectively. The mean $IC_{50}$ was 1.58 ± 0.14 $\mu M$.

The remaining 6 lines were all more sensitive to cisplatin, having $IC_{50}$s of between 0.66 $\mu M$ cisplatin and 0.83 $\mu M$ cisplatin. The mean $IC_{50}$ for this group was 0.69 ± 0.05 $\mu M$.

**DISCUSSION**

Cross-resistance of human tumors to cisplatin and radiation in vivo could be due either to inherent cellular resistance to both agents or to the tumor microenvironment which would affect tumor blood flow, possibly reducing cisplatin uptake and producing tumor hypoxia. Our results suggest that one major mechanism of cross-resistance to cisplatin and radiation is due to inherent cellular resistance to both agents. In early passage human tumor cell lines, which were not selected in vitro for cisplatin or radiation resistance, in vitro resistance to cisplatin and radiation was always accompanied by resistance to ionizing radiation. Although the numbers were small, it appears that this relationship was independent of cell type. The association between radiation and cisplatin resistance in vitro is particularly interesting because of the results of clinical trials using cisplatin-based regimens and radiotherapy in head-and-neck cancer. High correlations between response to cisplatin and subsequent response to radiation have been reported in patients receiving cisplatin-based regimens followed by radiotherapy (1, 2). Also, a high correlation between resistance to cisplatin and radiotherapy has been demonstrated in these studies.

Cross-resistance in the human tumor cells suggests a common mechanism underlying both radiation and cisplatin resistance. This finding is especially intriguing because the DNA lesions induced by both agents are different. Ionizing radiation produces primarily DNA single- and double-strand breaks (13), while cisplatin induces DNA-protein cross-links, DNA intrastrand and interstrand cross-links, and monofunctional adducts (14, 15).
Previous studies of ours have shown that resistance to radiation in human tumor cell lines is associated with an enhanced rate of rejoining of DNA double-strand breaks. By DNA elution analysis, radioresistant cells were shown to rejoin radiation-induced DNA double-strand breaks within 1 h of irradiation, while more sensitive cells required 2 to 4 h to rejoin induced DNA double-strand breaks. Defects in the ability to rejoin DNA double-strand breaks confer sensitivity to cisplatin as well as radiation. Studies with the radiosensitive, repair-defective Chinese hamster ovary cell mutant, xrs-5, suggest that this cell line is also sensitive to cisplatin as compared to its parental line CHO-K1 (Table 1). The xrs-5 cell line is defective in its ability to rejoin radiation-induced DNA double-strand breaks (16). These results suggest that the repair of radiation and cisplatin damage shares a common pathway, and therefore resistance to these two agents might reflect alterations in DNA repair.

In conclusion, there is a strong association between radiation and cisplatin resistance in early passage human tumor cell lines, suggesting that it is inherent resistance to these agents that underlies in vivo-observed cross-resistance.

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X-Ray and cis-Diamminedichloroplatinum(II) Cross-Resistance in Human Tumor Cell Lines

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