

# Inherent Sensitivity of Cultured Human Embryonal Carcinoma Cells to Adducts of *cis*-Diamminedichloroplatinum(II) on DNA<sup>1</sup>

Martin F. Pera,<sup>2</sup> Frank Friedlos, Judith Mills, and John J. Roberts

Radiotherapy Research Unit, Section of Radiotherapy [M. F. P., J. M.], and Molecular Pharmacology Unit, Section of Drug Development [F. F., J. J. R.], Institute of Cancer Research, Royal Cancer Hospital, Clifton Avenue, Sutton, Surrey SM2 5PX, United Kingdom

## ABSTRACT

Clinical and experimental data indicate that human embryonal carcinoma cells are unusually sensitive to the antitumor drug *cis*-diamminedichloroplatinum(II) (cisplatin), but the basis of this sensitivity is unknown. Using colony formation assays we measured survival of cultured human embryonal carcinoma cells following cisplatin treatment and related survival to the amount of platinum bound to DNA, determined by isolation of cellular DNA and flameless atomic absorption spectrometry, over a range of drug doses. Similar measurements were carried out on F9 mouse embryonal carcinoma cells and on a fibroblast cell strain from a patient with the genetic disease Fanconi's anemia, a syndrome associated with hypersensitivity to cytotoxic and clastogenic effects of difunctional DNA-binding agents. These results were compared with similar analyses on a variety of cultured cells from previous studies. The embryonal carcinoma cells and the Fanconi's anemia fibroblast strain were among the most sensitive cells on a dose-response basis. Since the amount of platinum bound to DNA after treatment of these cells was similar to values reported for many other cell types, it follows that mouse and human embryonal carcinoma cells are inherently sensitive to DNA-bound platinum adducts, to a degree approaching the sensitivity of fibroblasts from patients with xeroderma pigmentosum and Fanconi's anemia.

## INTRODUCTION

Many studies of the mechanism of action of anticancer drugs focus on drug resistance, in part because it is widely held that the emergence of drug-resistant subpopulations accounts for the failure to cure many common tumors with cytotoxic therapy (1). However, sensitivity and resistance are relative terms, and it may well be that most human tumors are not more sensitive at the cellular level to cytotoxic therapy than are normal proliferating cell populations. Surprisingly little is known regarding the mechanism of drug action in those few human tumors which may be cured with chemotherapy and which therefore may be truly considered to display a high degree of sensitivity to the drugs used. Analysis of how drugs actually succeed in curing such diseases might prove as informative for the design of new therapeutic approaches as analysis of why drugs fail.

Human testicular nonseminomatous germ cell tumors demonstrate remarkable sensitivity to cytotoxic therapy in the clinical setting. Combination chemotherapy with *cis*-diamminedichloroplatinum(II) (cisplatin), vinblastine or etoposide, and bleomycin can achieve cure rates in excess of 90% even in advanced disease (2, 3). Xenografts of human embryonal carcinomas in immunosuppressed mice showed more growth delay after treatment with maximally tolerated doses of cisplatin than other types of human xenograft tumors (4). Oosterhuis *et al.* (5) studied the response of several human and mouse embryonal carcinoma cell lines to cisplatin. These investigators showed that the germ cell tumor lines were more sensitive to this agent

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<sup>2</sup> To whom requests for reprints should be addressed.

than other cultured cell lines. The basis for this difference in response was not further explored.

Cisplatin is thought to exert its cytotoxic action through interaction with DNA (6, 7). By relating cell kill to binding of the drug to DNA over a wide range of doses, it is possible to determine whether cellular response to the drug is a function of the extent of drug uptake and binding to cell macromolecules, or whether response reflects inherent cellular sensitivity to DNA damage. Such studies have shown that cultured mammalian cells vary widely in their capacity to tolerate cisplatin-induced DNA damage (6, 7). Among human cell lines, fibroblasts from patients with the genetic disorders xeroderma pigmentosum (8) and Fanconi's anemia (9) show the highest degree of sensitivity to cisplatin; in the case of xeroderma pigmentosum it was shown that fewer platinum adducts on DNA were required to produce a given level of cytotoxicity in these cells than in normal human fibroblasts but such analyses have not been carried out in Fanconi's anemia cells. Here we show that human embryonal carcinoma cells are inherently sensitive to DNA damage induced by cisplatin, to a degree approaching the level of sensitivity seen in the genetic syndromes discussed above.

## MATERIALS AND METHODS

**Cell Lines.** The establishment and cell biological properties of the human germ cell tumor lines studied in this report will be described elsewhere.<sup>3</sup> All germ cell tumor lines came from previously untreated patients. GCT 27 and GCT 35 were derived from primary testicular teratocarcinomas, and GCT 46 was obtained from a lung metastasis of an embryonal carcinoma with yolk sac elements. F9 mouse embryonal carcinoma cells were a gift from Dr. Peter Goodfellow, of the Imperial Cancer Research Fund, London, United Kingdom. The Fanconi's anemia fibroblast cell strain was kindly provided by Dr. Francesco Gianelli of the Department of Pediatrics, Guy's Hospital Medical School, London, United Kingdom, and it was studied at passage levels 10-15.

**Cell Culture and Drug Treatment.** The human germ cell tumor lines were routinely cultured on plastic tissue culture vessels in monolayer in Dulbecco's Modified Eagle's medium supplemented with 10% fetal calf serum, 1  $\mu$ g/ml hydrocortisone, 2 mM glutamine, and penicillin plus streptomycin, on feeder layers of lethally irradiated mouse Swiss 3T3 cells as described elsewhere.<sup>3</sup> F9 cells and Fanconi's anemia fibroblasts were cultured in the same medium with the omission of hydrocortisone. For F9 cells, culture surfaces were pretreated with 0.1% gelatin in phosphate-buffered saline (180 mM NaCl, 3.4 mM KCl, 9.7 mM Na<sub>2</sub>HPO<sub>4</sub>, and 1.8 mM KH<sub>2</sub>PO<sub>4</sub>) in order to ensure attachment of the cells to the monolayer. Prior to survival and DNA-binding studies, cells were subcultured into fresh culture vessels in the absence of feeder cells at a 1:2 split ratio. Near-confluent cell monolayers were treated with cisplatin at various doses for 2 h at 37°C. In cell survival assays after drug treatment, cells were harvested with trypsin-EDTA (0.05% trypsin and 0.02% EDTA in phosphate-buffered saline) and washed in fresh medium twice; an inoculum sufficient to yield approximately 100 colonies was plated out onto 5-cm dishes with 2.5  $\times$  10<sup>5</sup> lethally irradiated mouse 3T3 cells (GCT 27, GCT 35, and GCT 46),

<sup>3</sup> M. F. Pera, M. J. Blasco Lafita, and J. M. Mills. Cultured stem cells from human testicular teratomas: the nature of human embryonal carcinoma, and its comparison with two types of yolk sac carcinoma, *Int. J. Cancer*, in press.

or  $2 \times 10^5$  lethally irradiated normal human fibroblast cells (Fanconi's anemia fibroblasts). F9 cells did not require feeder cell support. After a 14-day incubation period, the plates were fixed with methanol and stained with methylene blue, and the number of colonies with >50 cells was determined. Control colony-forming efficiencies were approximately 30, 10, and 5% for GCT 27, GCT 35, and GCT 46, respectively; 5% for Fanconi's anemia fibroblasts; and 60% for mouse F9 embryonal carcinoma cells.

**Determination of Platinum Bound to DNA.** Between  $5 \times 10^7$  and  $1 \times 10^8$  cells grown to near-confluence in 175-cm<sup>2</sup> culture flasks were harvested immediately following drug treatment. The DNA was extracted by previously described methods (10) using detergent cell lysis and proteinase K treatment, phenol extraction, precipitation from solution, RNase treatment, and reprecipitation, and then hydrolyzed in 0.1 M HCl. The nucleic acid base content of the hydrolysate was determined by measuring the absorbance at 260 nm, and the platinum content of the hydrolysate was measured by flameless atomic absorption spectrometry (10).

RESULTS

Fig. 1 shows the survival curves obtained following a 2-h exposure of F9 mouse embryonal carcinoma cells, Fanconi's anemia fibroblasts, and three human germ cell tumor cell lines to various doses of cisplatin. Each experiment was performed

at least twice. For most cell lines, cell survival was an exponential function of dose over at least three decades of killing; thereafter the curves sometimes flattened. Although such plateaus in survival curves were suggestive of a drug-resistant subpopulation, clones surviving high drug doses always displayed survival curves very similar to those of the parent cell lines (data not shown). The corresponding data for platinum binding to DNA for these same cell lines are shown in Fig. 2. Platinum binding to DNA was a simple linear function of drug dose over the dosage range studied.

From the data in Figs. 1 and 2, we calculated values for the slopes of the linear portions of the survival curves ( $D_0$ ), and the slopes of the curves relating Pt-DNA binding to drug dose, which yield an estimate of platinum bound to DNA per unit dose. By multiplying the  $D_0$  by the amount of platinum bound to DNA at a dose which reduces survival by 37% on the linear portion of the survival curve ( $B_0$ ) may be obtained. Table 1 shows these values for the cell lines studied in this report and a set of corresponding data from the literature, obtained in one of our laboratories using essentially similar techniques, for a range of cultured mammalian cells. Both sets of data are depicted in Fig. 3, which groups the cell lines into three categories: germ cell tumors; known hypersensitive cell types, including xeroderma pigmentosum fibroblasts, Fanconi's anemia fibro-

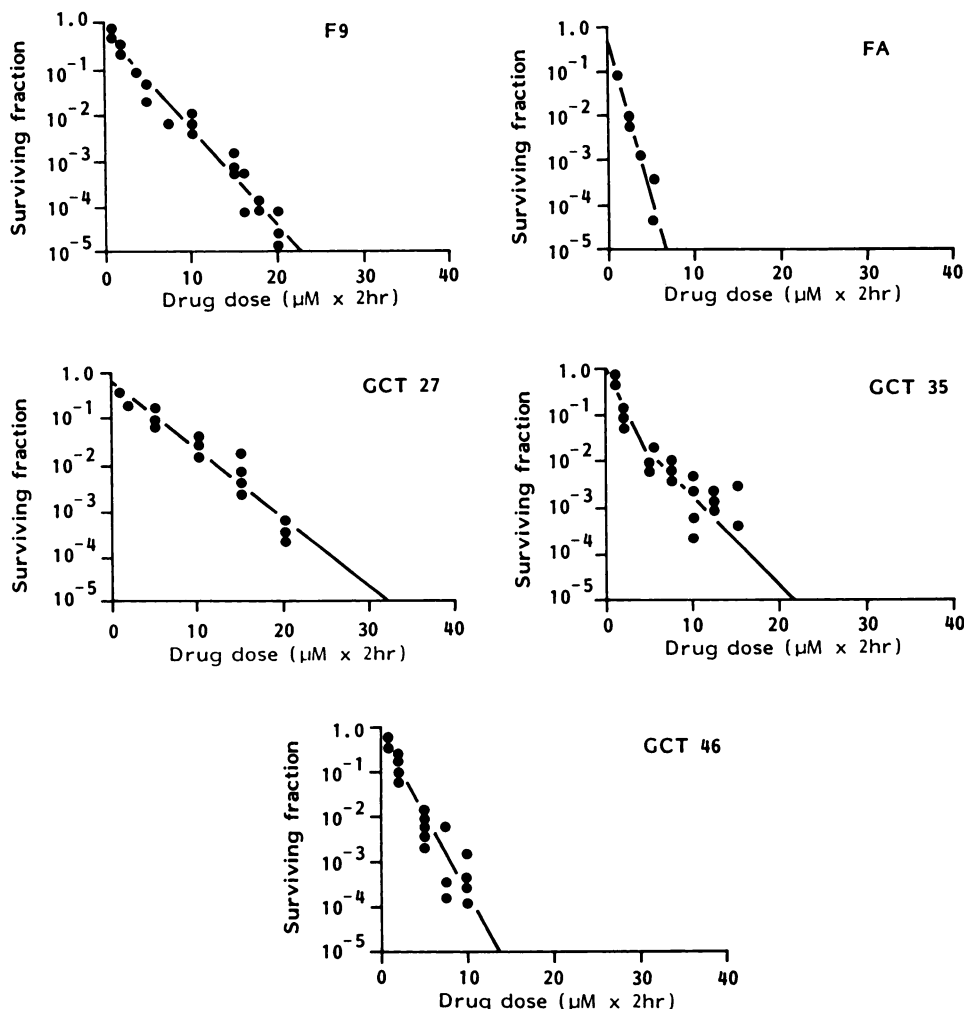


Fig. 1. Survival of germ cell tumor lines and Fanconi's anemia fibroblast strain versus cisplatin dose. Survival, colonies formed in treated cells/colonies formed in controls; drug dose, μM, 2-h drug exposure. FA, Fanconi's anemia.

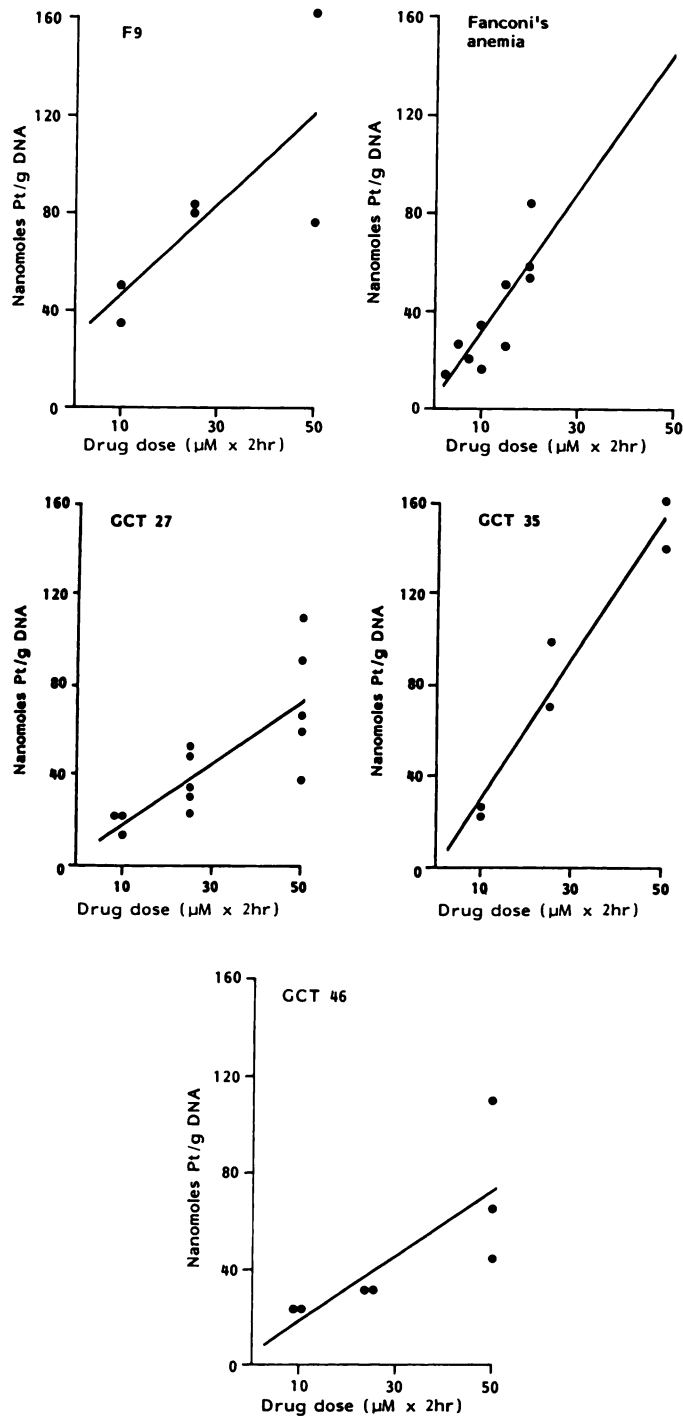


Fig. 2. Binding of platinum to DNA versus cisplatin dose. Binding nmol platinum/g DNA; drug dose,  $\mu\text{M}$ , 2-h drug exposure.

blasts, and the Walker rat carcinoma cell line (11, 12); and other cell types. Fig. 3 reveals three main points: (a) the germ cell tumor lines are among the most sensitive cell lines on a dose-response basis; (b) although there are indeed variations among cell types in the amount of platinum binding to DNA for a given drug exposure, there is no clear-cut relationship between this parameter and cellular sensitivity; (c) a consideration of the  $B_0$  values shows that the germ cell tumors clearly display an inherent sensitivity to platinum-induced DNA damage, to a degree approaching that of the three most sensitive cell types described thus far.

## DISCUSSION

Fibroblasts from patients with the genetic disorders xeroderma pigmentosum and Fanconi's anemia, as well as Walker rat carcinoma cells, show unusual sensitivity to cisplatin-induced DNA lesions. In the case of xeroderma pigmentosum, the cellular sensitivity phenotype extends to a wide variety of DNA-damaging agents and, for most of the genetic complementation groups, is related to a defect in DNA excision repair capacity (13). The molecular basis of the enhanced responsiveness of Fanconi's anemia cells and Walker rat carcinoma cells to cisplatin remains uncertain, but both cell types display a particular spectrum of sensitivity to the cytotoxic and clastogenic effects of a range of chemically diverse bifunctional cross-linking agents (11, 12). Attempts to relate this pattern of response to a specific defect in the overall repair of platinum lesions in DNA, or to a defect in the repair of DNA interstrand cross-links, have been unsuccessful.

In this study, we measured survival and DNA binding following treatment of human embryonal carcinoma cells with cisplatin. Available assays for total platinum binding to DNA do not permit measurement of binding at doses causing modest levels of cell killing in the highly sensitive cell lines studied here. It is clear, however, from these studies that at a given measurable level of platinum binding to DNA, far greater levels of cell killing are produced in embryonal carcinoma cells than in most other cell types. Thus these tumor cells are inherently sensitive to cisplatin damage in DNA.

Human nonseminomatous germ cell tumors are treated with combination chemotherapy, which often includes cisplatin, etoposide, and bleomycin. This report demonstrates that human germ cell tumor lines are inherently sensitive to platinum adducts on DNA; other studies in this laboratory have shown a similar enhanced sensitivity of these cells to etoposide and bleomycin.<sup>4</sup> Walker carcinoma cells and Fanconi's anemia cells do not show cross-sensitivity to these latter two agents (11, 12); therefore the phenotype of the germ cell tumor lines is somewhat different from these hypersensitive cells and from that of xeroderma pigmentosum.

At the molecular level, cisplatin, etoposide, and bleomycin can all produce DNA damage involving both strands of the double helix. Cisplatin produces DNA interstrand cross-links (6), etoposide forms complexes with double-stranded DNA and topoisomerase II (14), and bleomycin produces double-strand breaks in DNA (15). Because of the nature of such DNA damage, it seems likely that recombinational mechanisms might play a role in its repair and it is tempting to speculate that human embryonal carcinoma cells do not express some aspect of recombinational repair. There is evidence that the differentiated derivatives of teratocarcinoma cells are more resistant to cisplatin than the tumor stem cells (5, 16, 17); therefore it seems possible that the expression of this putative recombinational repair pathway is developmentally regulated. For example, the recent demonstration that the maternally and paternally derived portions of the genome perform separate and distinct functions during development (18) might imply that mitotic recombination could result in loss of alleles critical to growth in embryonic cells. Thus we suggest that the inherent sensitivity of germ cell tumors to DNA damage is a function of the developmental state of the tumor stem cells, and a reflection of the properties of the normal embryonic cells to which they correspond, rather than a unique transformation-related prop-

<sup>4</sup> J. I. Delic, M. F. Pera, F. Friedlos, and J. J. Roberts, unpublished observations.

Table 1 Relationship between cell survival after cisplatin treatment and platinum binding to DNA in human embryonal carcinoma cells and other cell types

Cell line	$D_0$ ( $\mu\text{M}$ for 2 h) <sup>a</sup>	Platinum bound to DNA/unit dose (nmol Pt/g DNA/ $\mu\text{M}$ for 2 h)	$B_0$ (nmol Pt/g DNA)	Ref.
F9	2.0	1.9	3.8	This study
Fanconi's anemia, fibroblast strain	0.50	2.9	1.4	This study
GCT27	2.9	1.4	4.0	This study
GCT35	1.1	3.1	3.4	This study
GCT46	1.0	1.3	1.3	This study
Normal human fibroblast (FL/G)	2.5	3.0	7.5	20
Normal human fibroblast (FL/A)	3.2	2.3	7.4	8
V79 Chinese hamster cells	7.5	1.1	8.5	21
B16 mouse melanoma cells	<sup>b</sup>	<sup>b</sup>	12.0	22
Walker rat carcinoma cell (cisplatin resistant)	8.3	1.2	10.0	12
Walker rat carcinoma cell (wild-type)	0.5	1.5	0.8	12
Xeroderma pigmentosum fibroblast (XP12BE)	2.2	0.8	1.8	8

<sup>a</sup>  $D_0$ , dose required to reduce survival from  $n$  to  $0.37 n$  on the exponential portion of the survival curve;  $B_0$ , amount of platinum bound to DNA required to reduce survival from  $n$  to  $0.37 n$  on the exponential portion of the survival curve.

<sup>b</sup> Treatment *in vivo*.

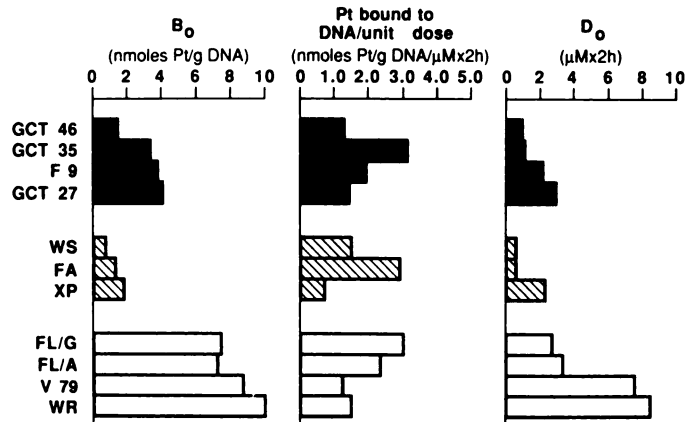


Fig. 3.  $D_0$ , amount of platinum bound to DNA per unit dose, and  $B_0$  values for germ cell tumors (■), known hypersensitive cells (▨), and other cell types (□). Each column corresponds to one cell line; left to right, GCT46 (human embryonal carcinoma), GCT35 (human embryonal carcinoma), F9 (mouse embryonal carcinoma), GCT27 (human embryonal carcinoma), wild-type Walker rat carcinoma (WS), Fanconi's anemia fibroblast strain (FA), xeroderma pigmentosum fibroblast strain (XP), normal human fibroblast (FL/G), normal human fibroblast (FL/A), V79 (Chinese hamster lung cell line), subline of Walker rat carcinoma with normal sensitivity to difunctional alkylating agents (WR).

erty. An analogous situation exists with lymphoid malignancy; normal cells in lymphocyte ontogeny which certain tumor stem cells most nearly resemble are also exquisitely sensitive to corticosteroids or deoxycoformycin (19). Successful cancer chemotherapy may rely more on cell type specificity instead of broad spectrum antitumor activity.

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