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Morphological change and cellular differentiation induced by cisplatin in human neuroblastoma cell lines

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Summary. In 1986 we reported on the capacity of *cis*-diamminedichloroplatinum(II) (cisplatin, CDDP) to induce erythroid cellular differentiation in the K562 cell [9]. To continue our study of the differentiating activity of cisplatin, we treated two human neuroblastoma cell lines with different doses of the drug in vitro. Both cell lines showed changes in morphology; however, only one achieved a fully differentiated neuronal phenotype (cisplatin concentration 1 μg/ml). The differentiated neuroblastoma cells exhibited extensive neurite outgrowth that reached maximal elongation after 5 days of culture, forming several interconnections. Cisplatin could induce neuronal differentiation, as did retinoic acid, a neuroblastoma-differentiating agent. The results show that cisplatin should be a candidate for further in vitro and in vivo studies of induced differentiation.

Introduction

Cisplatin, *cis*-diamminedichloroplatinum(II), is an anticancer drug used in the treatment of solid tumors. High doses of cisplatin are used in the management of human neuroblastoma (NB), a tumor frequently arising in pediatric patients [2]. In studies on the mechanisms of the antitumor activity of cisplatin, we have demonstrated that cytostatic doses of this drug induce erythroid differentiation in K562, a human erythroleukemia cell line [9]. We report the results of our studies on the effects of cisplatin on the differentiation of human NB cells in vitro.

Materials and methods

Two NB cell lines endowed with different morphology have been used: SK-N-BE(2)C, with intermediate(I)-type morphology, and LA1-15n, with neuroblastic (N)-type morphology [3]. The cells were cultured in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 15% fetal calf serum and treated with cisplatin (Bristol-Myers Company) at 0 and 48 h at final concentrations of 8, 4, 2, 1 and 0.5 μg/ml or with 10^{-6} *M* retinoic acid (RA, Sigma Chemical Co., St. Louis). The differentiation of the NB cell lines produced by the drugs was evaluated by optical phase-contrast microscopic observation of changes in the morphology of the cells as well as the formation of long neurite extensions. Drug toxicity, depending on the concentration in the medium, was assayed by [^{3}H]-thymidine uptake and by determining cellular viability by the trypan blue exclusion test. The HSAN 1.2 monoclonal antibody [4] was used to monitor the expression of specific NB-associated antigens during treatment with cisplatin or RA. The cells were analyzed for green fluorescence with an Epics Profile Analyzer (Kontron Instrument).

Results and discussion

RA consistently induced differentiation in NB cell lines, as shown by the results reported in Fig. 1, confirming previously published observation [8]. SK-N-BE(2)C cells showed a good differentiative response following exposure to 1 μg/ml cisplatin; the neurite extension reached a maximum after 5 days of culture (Fig. 1). By this time, >95% of the cells displayed a clearly differentiated phenotype. Cisplatin concentrations of >1 mg/ml were toxic over a 5-day incubation period. The toxicity of the drug, depending on the concentration in the medium, is represented in the dose-response curves in Fig. 2. SK-N-BE(2)C cells responded to 0.5 μg/ml drug by becoming enlarged and displaying additional nuclei and nucleoli, but few, if any, neurite extensions were induced by such treatment. In contrast, the LA1-15n cell line did not fully differentiate in response to cisplatin at doses of 1 or 0.5 μg/ml; however, these cells became flat and larger (Table 1). The expression of the HSAN 1.2 antigen was not affected in either cell line by cisplatin during the 1st 7 days of treatment (Table 2). In both cell lines, <5% of cells showed spontaneous neurite formation during a 10-day period of observation.

In conclusion, cisplatin has different effects on the human NB cell lines. The SK-N-BE-(2)C cells showing intermediate morphology, responded to 1 μg/ml cisplatin by manifesting neurite outgrowth and several interconnections. Very similar morphological changes occur during RA-induced differentiation. In contrast, the LA1-15n cells became larger and flater in response to cisplatin, appearing more like epithelial cells and they did not extend

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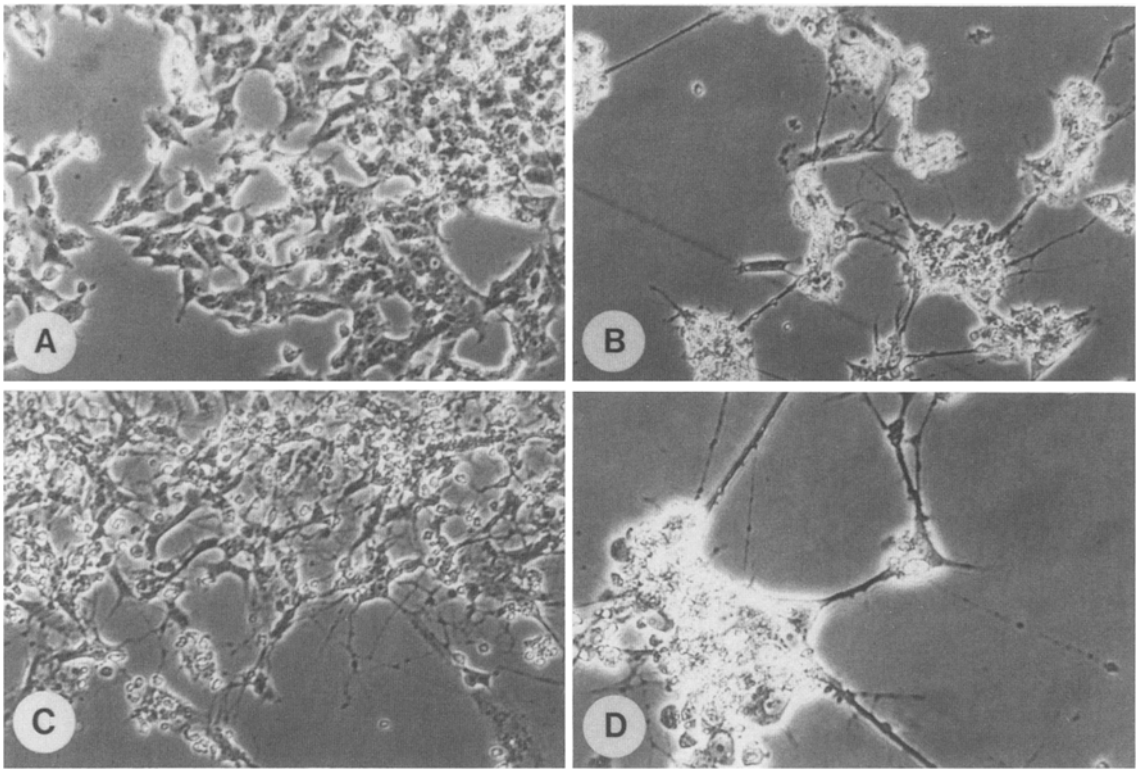

Fig. 1. Phase-contrast optical microscopy of **A** an untreated cell (×160), **B** an RA-treated cell (×160), **C** cisplatin-treated cell (×160), **D** cisplatin-treated cell (×320)

neuritic processes. Thus, our results extend previous observations by Rettig et al. [3] on the naturally ocurring in vitro conversion of NB cells from a neuroblast-type morphology to an epithelial-like (S)-type of morphology; cisplatin seems to be capable of inducing the transdifferentiation N/S in the LA1-15n cell line.

The expression of the NB-associated antigen recognized by the HSAN 1.2 monoclonal antibody did not change in either cisplatin- or RA-treated cells (data not shown). The HSAN 1.2 antibody resulted from mouse plasmocytoma cells that were immunized with SMS-SAN human NB cells [4]; it was introduced to detect a specific antigen expressed by NB but not by other morphologically small-cell tumors. Very few data concerning the expression of HSAN 1.2 during NB cellular differentiation are available. Our results suggest that the levels of its expression do not correlate with NB differentiation and do not seem to depend on the maturity of the cell.

The present data show a differentiating activity of cisplatin that induces morphological alteration characteristic of the mature neuronal phenotype in human NB

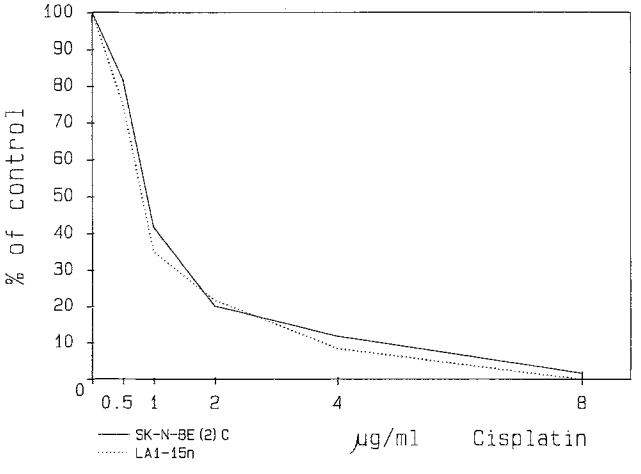

Fig. 2. Dose-response curve of [^{3}H]-thymidine uptake after 5 days treatment with cisplatin in SK-N-BE(2)C and LA1-15n cells

Table 1. Morphological effect of different doses (µg/ml) of cisplatin on SK-N-BE(2)C and LA1-15n cell lines

Cell line	8	4	2	1	0.5
SK-N-BE(2)C	TOX	TOX	TOX	NEURITE	LARGE
LA1-15n	TOX	TOX	TOX	L/F	L/F

TOX, toxic dose, NEURITE, neurite outgrowth with elongation; L/F, cell became large and flat

Table 2. Expression of related neuroblastoma antigen during cisplatin treatment (1 µg/ml)

Cell line	Days of culture:			
	0	1	2	7
SK-N-BE(2)C	67±0.22[a]	65±0.18	60±1.0	66±1.8
LA1-15n	98±0.20	99±0.21	92±1.3	96±2.1

[a] The result express the mean ±SD of the percentage of HSAN 1.2-positive cells from three different experiments

cells. Several other anticancer drugs can induce terminal cell differentiation in vitro or in vivo in a variety of cells, and some of them are used in clinical trials [1, 5, 6, 7, 10]. Our results indicate that cisplatin may have important applications not only as a cytoreductive agent but also in differentiating therapeutic protocols.

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