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ORIGINAL ARTICLE

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Synergistic effects of ICI 182,780 on the cytotoxicity of cisplatin in cervical carcinoma cell lines

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Abstract *Purpose*: We investigated the ability of the novel pure antiestrogen ICI 182,780 to modulate the cytotoxic effects of cisplatin in several cervical cancer cell lines. *Methods*: The effect of cisplatin alone and cisplatin combined with ICI 182,780 on cellular death was studied using an assay based on a tetrazolium dye (sodium 3′-[1-(phenylamino-carbonyl)-3,4-tetrazolium], XTT). Before and after treatment with ICI 182,780, expression of the estrogen and progesterone receptor genes were assessed by a reverse transcriptase polymerase chain reaction (RT-PCR). Cell-cycle modifications after combined treatment with cisplatin and ICI 182,780 were studied by flow cytometry. *Results*: Analysis of the data by the isobologram method showed that the combination of ICI 182,780 and cisplatin produced a synergistic antiproliferative effect in cervical cancer cells. The effect of ICI 182,780 on the cytotoxicity of cisplatin could be mediated, at least partially, by inhibition of estrogen and progesterone gene expression and by arresting the cell cycle at the G_2/M phase. *Conclusions*: Our results suggest that ICI 182,780 can improve the efficacy of cisplatin in cancer cells and that this antihormonal drug therapy may be a useful candidate for further evaluation in combination with antineoplastic drugs, particularly cisplatin, in the treatment of cancer.

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Introduction

Cisplatin and its derivatives are important drugs in cancer therapy. It has been widely used for its potent cytotoxic effects upon a variety of tumor types including testicular, ovarian, and cervical carcinoma [2, 12, 26, 30]. However, the administration of cisplatin is associated with serious side effects, including nephrotoxic and neurotoxic events [14]. Frequently, cisplatin is administered in combination with other drugs [14]. There have been several studies aimed at finding drugs able to potentiate the antiproliferative effect of cisplatin without increasing the already serious side effects, but the search has not been successful.

Among the chemosensitizer drugs, antiestrogens such as tamoxifen and ICI 182,780 have been used to modulate the cytotoxic activity of antineoplastic agents such as doxorubicin and paclitaxel. Resistance to these drugs usually develops with the multidrug-resistance (MDR) phenotype associated with participation of the drug-efflux pump P-glycoprotein (Pgp), a 170-kDa membrane protein in MDR cells [10]. On the other hand, some studies have shown that tamoxifen sensitizes the effect of cisplatin in several cell lines. For example, it has been demonstrated that in head and neck squamous cell carcinoma, tamoxifen alone induces a transient G_1 arrest that greatly sensitizes the cells to apoptosis induced by cisplatin [36]. Recently, it has also been demonstrated that tamoxifen increases the apoptotic effect of cisplatin in primary endometrial cell cultures [11]. Therefore, it has been concluded that tamoxifen can enhance the antitumor activity of cisplatin in a preclinical model through apoptosis. Following these results, a phase I trial of tamoxifen in combination with cisplatin in patients with lung cancer was done, and it was concluded that a regimen of high-dose tamoxifen in

combination with cisplatin can be safety administered, since it did not show any hematological toxicity [31].

Tamoxifen has been commonly used for all stages of breast carcinoma. However, several authors have established an association between the use of tamoxifen in breast cancer and the subsequent development of endometrial carcinoma [13, 28, 35]. Moreover, it has been shown that tamoxifen at a low concentration (10^{-9} *M*) causes stimulation of cell proliferation in a cervical cancer cell line (SFR cell line) that does not contain the estrogen receptor (ER) [23]. The novel pure antiestrogen, ICI 182,780, may have advantages over tamoxifen because it is devoid of estrogen agonist activity and its affinity with the ER is approximately 100 times greater than that of tamoxifen [20]. It has been used in postmenopausal patients with breast cancer, and clinical results have confirmed that it has useful activity against breast cancer [9], as well as a safety profile that includes reduced adverse effects such as headache and nausea [37]. Additionally, there is no evidence of agonist activity in the endometrium of postmenopausal women [1]. Consequently it has been increasingly used against breast cancer [8].

The aim of this study was to investigate whether ICI 182,780 in combination with cisplatin could act synergistically in human cervical cancer cells. In an effort to correlate the mechanism of action of this antiestrogen in modulating cisplatin activity, the effects of ICI 182,780 on ER and progesterone receptor (PR) gene expression was analyzed. Finally, the cell-cycle modifications induced by cisplatin in cell lines in both the presence and the absence of ICI 182,780 were studied.

Materials and methods

Drugs

Cisplatin (Sigma, St. Louis, Mo.) was reconstituted in distilled water immediately before use. ICI 182,780 (Tocris Cookson, Balwin, Mo.) was reconstituted in absolute ethanol (stock solution) and kept at −20°C until use. All reagents were purchased from Sigma and Gibco-BRL (Gaithersburg, Md.). Taq DNA polymerase was purchased from Perkin-Elmer (Branchburg, N.J.).

Cell cultures

The HeLa, SiHa, and CaSki human cervical cancer cell lines and the MCF-7 human breast cancer cell line were used. They were obtained from ATCC (Rockville, Md.). All cell lines were routinely maintained as a monolayer in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (Gibco BRL), and incubated at 37°C in an atmosphere comprising 5% CO_2 and 95% air at high humidity. Cells were harvested with 0.025% trypsin (Sigma) and 0.01% EDTA (Gibco BRL).

Growth inhibition experiments

The effect of ICI 182,780 on proliferation of cells exposed to cisplatin was evaluated using the XTT assay (sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis; Roche Molecular Biochemicals) [4]. The assay is based on the cleavage of the yellow tetrazolium salt XTT to form an orange formazan dye by metabolically active cells. The procedure was as follows. Cells were seeded into 96-well plates (Costar Cambridge, Mass.) at a density 2×10^3 viable cells per well in 100 µl phenol red-free culture medium previously treated with dextran-coated charcoal. At the end of the treatment with cisplatin alone or the combination of cisplatin plus ICI 182,780, 50 µl XTT was added to each well (final concentration 0.3 mg/ml), followed by incubation for 4 h in a humidified atmosphere containing 5% CO_2 at 37°C. The absorbance of the samples was measured spectrophotometrically at 492 nm using a microtiter plate ELISA reader.

Treatments with ICI 182,780 and cisplatin

The cells were conditioned for 4 days with 0.1, 1.0 or 10 µ*M* ICI 182,780. The final volume was 100 µl per well. Control cells were exposed only to vehicle (the final ethanol concentration never exceeded 1% in treated and control samples). At the end of the exposure period, the culture medium was removed and fresh medium with various amounts of cisplatin (0.1–330 µ*M*) was added for 4 h. After simultaneous and individual exposure to the drugs, the cells were cultivated for 24 h. Cell proliferation was evaluated using the XTT assay. The mean concentration in each set of three or four wells was determined in triplicate. The percentage growth inhibition was calculated and IC_{50} values (concentration of drug to achieve 50% growth inhibition) were obtained graphically from the survival curves.

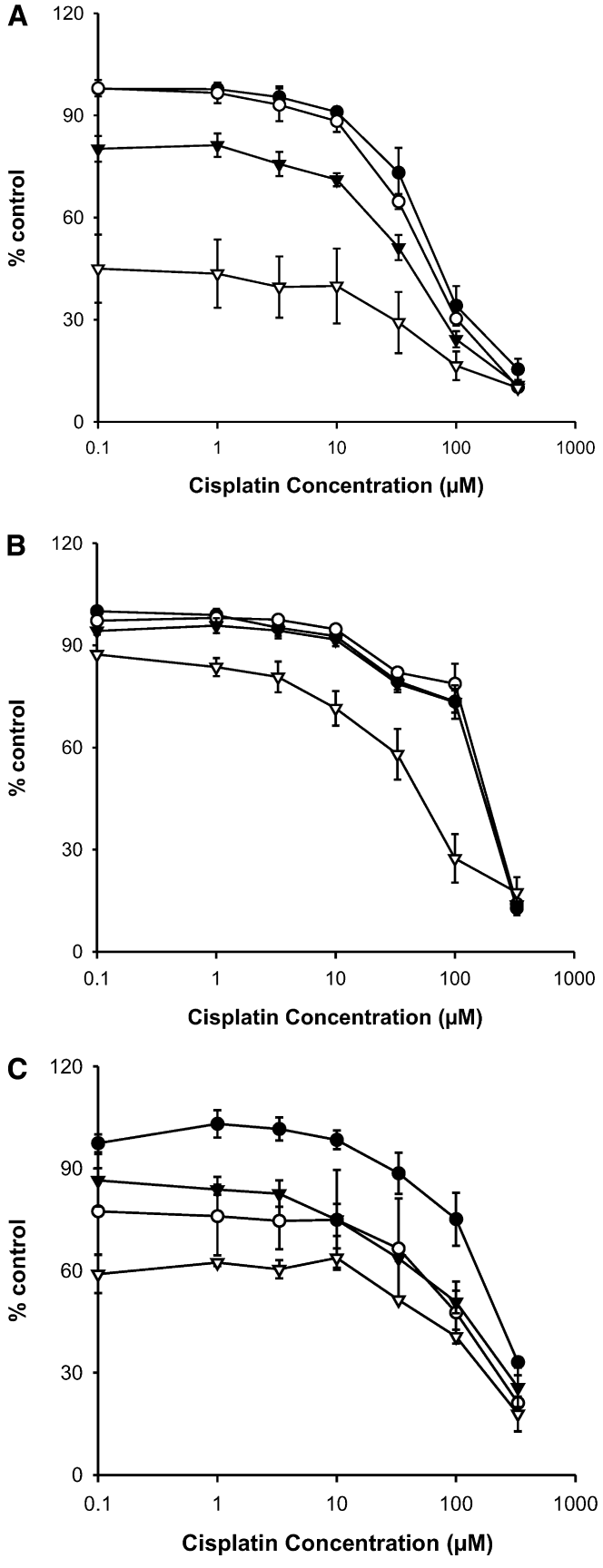
Data analysis of drug combination

Synergism or additivity was determined by calculating the combination index (CI) using de equation: $CIx = (D_1/Dx_1) + (D_2/Dx_2) + \alpha(D_1)(D_2)/(Dx_1)(Dx_2)$, where CIx represents the CI value for x% effect, Dx_1 and Dx_2 represent the doses of agents 1 and 2 required to exert x% effect alone, and D_1 and D_2 represent the doses of agents 1 and 2 that elicit the same x% effect in combination with the other agent, respectively. The factor α indicates the type of interaction: $\alpha = 0$ for mutually exclusive drugs (similar mechanisms of action), and $\alpha = 1$ for mutually non-exclusive drugs (independent modes of action) [6, 33]; the equation was resolved for $\alpha = 1$. A CI of 1 indicates additivity, a CI of < 1 synergism and a CI of > 1 antagonism.

RT-PCR analysis

After exposure to ICI 182,780 (10 µ*M*), the total RNA was isolated from each cell line with a single-step method based on guanidine isothiocyanate/phenol/chloroform extraction using TRIzol reagent (Gibco BRL). The RNA concentration was determined by absorbance at 260 nm and its integrity was verified by electrophoresis on 1.1% denaturing agarose gels in the presence of 2.2 *M* formaldehyde. Total RNA was reverse-transcribed to synthesize single-strand cDNA. The RT reaction mix (10 µl) was subjected to PCR to simultaneously amplify ER and PR gene isoforms, and the *β*-actin gene as internal control. The sequences of the specific primers unique for ER were 5′-GGA GAC ATG AGA GCT GCC AAC-3′ (sense) and 5′-CCA GCA TGT CGA AGA TC-3′ (antisense). The primers used for common PR-A + B were 5′-gct acg aag tca aac cca gt-3′ (sense) and 5′-cac cat ccc tgc caa tat c-3′ (antisense). The 50 µl PCR reaction included: 10 µl of previously synthesized cDNA, 20 m*M* Tris-HCl (pH 8.3), 50 m*M* KCl, 1 m*M* $MgCl_2$, 0.2 m*M* of each dNTP, 0.5 µ*M* of each primer, and 2.5 U Taq DNA polymerase. Negative controls without RNA and with non-retrotranscribed RNA were included in all experiments.

After an initial denaturation step at 95°C for 5 min, the PCR reaction was performed for 30 cycles. The cycle profiles for ER, PR and *β*-actin gene amplification were: 95°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The number of cycles performed was



◄

Fig. 1A–C Representative growth inhibition curves of HeLa (**A**), SiHa (**B**) and CaSki (**C**) cells exposed to cisplatin alone (●) and in combination with ICI 182,780 at 0.1 μ*M* (○), 1 μ*M* (▼) and 10 μ*M* (▽). Cells were exposed to ICI 182,780 for 4 days followed by cisplatin for 4 h. After treatment, the effect was evaluated at 24 h. All growth inhibition assays were repeated in triplicate in at least three independent experiments. Values are the means ± SEM

within the exponential phase of the amplification process. All PCR products were always studied and analyzed together in all experiments. PCR products (25 μl) were separated on 2% agarose gel and stained with ethidium bromide. ER, PR and *β*-actin absorbance was analyzed by densitometry using the NIH Image 1.62 program. Three independent experiments were performed for each cell line.

Cell cycle analysis

The cells were synchronized and plated 2×10^5 cells/ml in specific medium. After 24 h the medium was replaced with fresh medium containing ICI 182,780 alone or in combination with the IC_{25} concentration of cisplatin following the protocol previously described. When this process was completed the cultured cells were harvested and washed twice with phosphate-buffered saline, then fixed with 70% (v/v) ethanol. The samples were concentrated by removing ethanol and by treating with 1% (v/v) Triton X-100 and 0.01% RNase for 10 min. Cellular DNA was stained with 0.05% propidium iodide for 20 min at 4°C. Following flow cytometry, acquiring a minimum of 2×10^5 nuclei, analysis was performed with the MultiCycle software package (Modfit v. 2.01; Verity Software House, Topsham, Me.). At least three independent experiments were performed for each cell line.

Results

Growth inhibition experiments

Cytotoxicity is expressed as percentage growth inhibition of HeLa, SiHa and CaSki cells treated with cisplatin for 4 h, or cisplatin in combination with ICI 182,780 pretreatment. Cell growth was evaluated after 4 days of exposure to ICI 182,780 (Fig. 1). The antiproliferative effect of cisplatin (0.1–330 μ*M*) was potentiated in combination with ICI 182,780 (1.0 and 10 μ*M*) in HeLa cells (Fig. 1A). In SiHa cells, a synergistic effect was observed only at the highest concentration of ICI 182,780 tested (10 μ*M*; Fig. 1B). However, in CaSki cells, ICI 182,780 at 0.1, 1 and 10 μ*M* increased the sensitivity to the antiproliferative effect of cisplatin, but the effect was not dose-dependent (Fig. 1C).

Conditioning with ICI 182,780 at 0.1, 1.0 and 10 μ*M* for 4 days alone did not significantly inhibit the growth of CaSki and SiHa cells (the effect at the highest dose of 10 μ*M* was 16% and 10%, respectively), whereas ICI 182,780 at 10 μ*M* inhibited the growth of HeLa cells by 52% (Fig. 2). The IC_{50} of cisplatin in combination with ICI 182,780 was lower than that of cisplatin alone in HeLa, SiHa and CaSki cells (Table 1). The effect was more evident in HeLa cells, with an approximately 18 times higher potency. SiHa and CaSki cells were more resistant to cisplatin alone, but the combination also resulted in a clear potentiation of up to 4.5-fold.

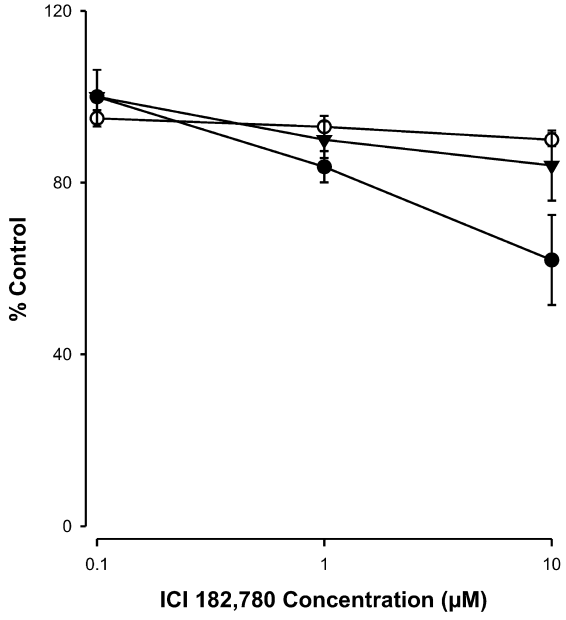


Fig. 2 Representative growth inhibition curves of HeLa (●), SiHa (○), and CaSki (▼) cells exposed to ICI 182,780 at different concentrations for 4 days. Values are the means ± SEM

Table 1 Effect of pretreatment with ICI 182,780 on the sensitivity of human cervical cell lines to cisplatin. Cells were pretreated or not pretreated with ICI 182,780 (10 μ*M*) for 4 days before exposure to cisplatin for 4 h. Values are the means of triplicate determinations in at least three independent experiments

Cell line	Cisplatin IC_{50} (μ*M*)	
	Cisplatin alone	Cisplatin + ICI 182,780
HeLa	63	< 3.5
SiHa	175	41
CaSki	220	49

Evaluation of the drug combination

To determine whether the combination effect of ICI 182,780 and cisplatin was synergistic or additive, the CI was determined using the equation given in Materials and methods. The CI obtained showed that the interaction of ICI 182,780 and cisplatin was synergistic throughout range of drug doses tested. The data are summarized in Table 2.

ER and PR gene expression in cells treated with ICI 182,780

RNA from MCF-7 cells treated with ICI 182,780 or untreated served as control. In untreated HeLa, SiHa and CaSki cells, ER and PR gene expression levels were lower than in MCF-7 cells. However, a positive band was found after 40 cycles of amplification in all cells. In HeLa and SiHa cells treated with ICI 182,780, ER gene expression was undetectable, whereas in CaSki cells expression was not changed (Fig. 3). Expression levels of PR mRNA were moderately decreased in the three ICI 182,780-treated cell lines (Fig. 4). The results were evaluated semiquantitatively by calculating the ratios of the expression of the ER or PR gene to that of β-actin.

Cell cycle assays

The cell cycle distribution of cells treated with cisplatin alone or in combination with ICI 182,780 (10 μ*M*) is shown in Table 3. Untreated control cells showed progressive accumulation in G_0/G_1 with a parallel decrease in the population in S and G_2/M. However, with the combined drug treatment, the population distribution changed: in HeLa cells, a significant increase in the G_2/M population and a significant decrease in the S phase population were observed. The values for SiHa cells remained lower than those of the control, but the difference did not reach statistical significance, whereas in CaSki cells G_0/G_1, S and G_2/M values were similar to those observed in control cells. The ratio between cells in the G_2 phase and those in the G_1 phase was calculated. The combination was more effective in HeLa cells.

Discussion

Cisplatin-based chemotherapy achieves a complete response in 70–80% of patients with germ cell tumors. However, only a minority of patients in whom first-line regimens have failed respond to an aggressive regimen [7]. These results have motivated the search for new agents or new regimens for cisplatin combinations with the purpose of increasing antitumoral activity and decreasing secondary events. In vitro chemosensitivity tests at the laboratory level are perhaps the most frequently employed tools in combination analysis. They provide the basis for clinical trial protocol design for cancer treatment.

In this work, using in vitro assays, it was shown that a new antiestrogen, ICI 182,780, combined with cisplatin was able to enhanced cytotoxicity in three cervical cancer cell lines (HeLa, SiHa and CaSki). These cervical cancer cell lines were chosen for the study because they contain the human papillomavirus (HPV) type 18 (HeLa) and type 16 (SiHa and CaSki) genotypes, which have been shown in multi-institutional studies to account for > 65% of all HPV DNA-positive invasive cervical carcinomas. Moreover, they represent different histological subtypes: HeLa cells were derived from an adenocarcinoma, SiHa cells from a squamous carcinoma, and CaSki cells from an epidermoid cervical carcinoma.

In accordance with the findings of De Vincenzo et al. [10], in MCF-7 cells and their doxorubicin-resistant variant (MCF-7 ADR), ICI 182,780 increases the effect of doxorubicin, suggesting the participation of the Pgp drug-efflux pump. In this investigation, cisplatin was

Table 2 Synergistic antiproliferative effects of combinations of ICI 182,780 and cisplatin in HeLa, SiHa and CaSki cells. The combination index was calculated according to the equation given in the text, and CIx, D_1, D_2, Dx_1 and Dx_2 are as defined in the text

Cell line	Doses for x% effect (μ*M*)				Control growth (x%)[a]	Combination index (CIx)[b]	Interaction
	Drugs in combination		Drugs alone				
	Cisplatin (D_1)	ICI 182,780 (D_2)	Cisplatin Dx_1)	ICI 182,780 (Dx_2)			
HeLa	0.1	1	21	1.3	80	0.79	Synergistic
	1.0	1	19	1.2	81	0.90	Synergistic
	3.3	1	25	1.7	76	0.71	Synergistic
	10	1	31	2.4	71	0.74	Synergistic
	33	1	64	> 10	51	0.52	Synergistic
	100	1	161	> 10	24	0.63	Synergistic
	330	1	> 330	> 10	11	0.11	Synergistic
	0.1	10	79	> 10	45	0.10	Synergistic
	1.0	10	82	> 10	43	0.11	Synergistic
	3.3	10	93	> 10	40	0.13	Synergistic
	10	10	92	> 10	40	0.21	Synergistic
	33	10	131	> 10	29	0.35	Synergistic
	100	10	243	> 10	17	0.49	Synergistic
	330	10	> 330	> 10	10	0.11	Synergistic
SiHa	0.1	10	35	15	87	0.60	Synergistic
	1.0	10	49	16	84	0.40	Synergistic
	3.3	10	59	26	81	0.33	Synergistic
	10	10	93	36	72	0.20	Synergistic
	33	10	144	> 100	58	0.32	Synergistic
	100	10	268	> 100	27	0.47	Synergistic
	330	10	310	> 100	17	1.16	
CaSki	0.1	0.1	86	15	77	0.007	Synergistic
	1.0	0.1	91	17	76	0.017	Synergistic
	3.3	0.1	98	18	74	0.039	Synergistic
	10	0.1	97	18	75	0.108	Synergistic
	33	0.1	134	29	67	0.249	Synergistic
	100	0.1	233	> 100	48	0.430	Synergistic
	330	0.1	> 330	> 100	21	< 1.0	
	0.1	1	50	7.4	86	0.13	Synergistic
	1.0	1	60	9.3	84	0.12	Synergistic
	3.3	1	65	10.5	83	0.14	Synergistic
	10	1	97	17.9	75	0.16	Synergistic
	33	1	148	34.7	63	0.24	Synergistic
	100	1	215	> 100	51	0.48	Synergistic
	330	1	> 330	> 100	26	0.10	Synergistic
	0.1	10	171	47	59	0.24	Synergistic
	1.0	10	154	38	62	0.27	Synergistic
	3.3	10	163	42	60	0.30	Synergistic
	10	10	148	35	64	0.35	Synergistic
	33	10	211	> 100	51	0.25	Synergistic
	100	10	277	> 100	41	0.46	Synergistic
	330	10	> 330	> 100	18	< 1.0	

[a]Mean values of three separate experiments performed in triplicate

[b]CI = 1 indicates additivity, CI < 1 synergism and CI > 1 antagonism

chosen because it is not a substrate for Pgp [29]. In addition, our intention was to determine the activity of the drug combination in a type of cancer, cervical cancer, that does not respond to hormonal treatment. As far as we know, there are no data indicating an effect of a pure antiestrogen in cervical cancer.

Although the normal cervix is known to respond to steroid sex hormones, hormonal treatments are not frequently employed in cervical carcinoma therapy. Indeed, this carcinoma is traditionally considered not to respond to antihormonal therapy [34]. This tumor exhibits low or undetectable levels of ER and PR as determined by immunohistochemical and ligand-binding assays [15, 38]. In the cervical cancer cell lines used in this investigation, ER and PR gene levels were relatively low compared to those observed in MCF-7 cells. These findings are in accordance with clinical data.

Despite the fact that the levels of ER and PR gene expression in cervical cancer were low, the levels were sufficient to demonstrate that ICI 182,780 downregulated ER and PR genes in HeLa and SiHa cells, indicating a ligand-receptor interaction. It is probable that these effects were partially responsible for cisplatin sensitization in HeLa and SiHa cells, which are the most sensitive and the most resistant cervical cancer cell lines respectively.

The highest effect in HeLa cells, which are adenocarcinoma cells, may also be related to the fact that adenocarcinomas are more likely to be hormonally sensitive. Interestingly, it has been reported recently that estrogens may induce many physiological effects at the membrane level, such as DNA synthesis and cyclin

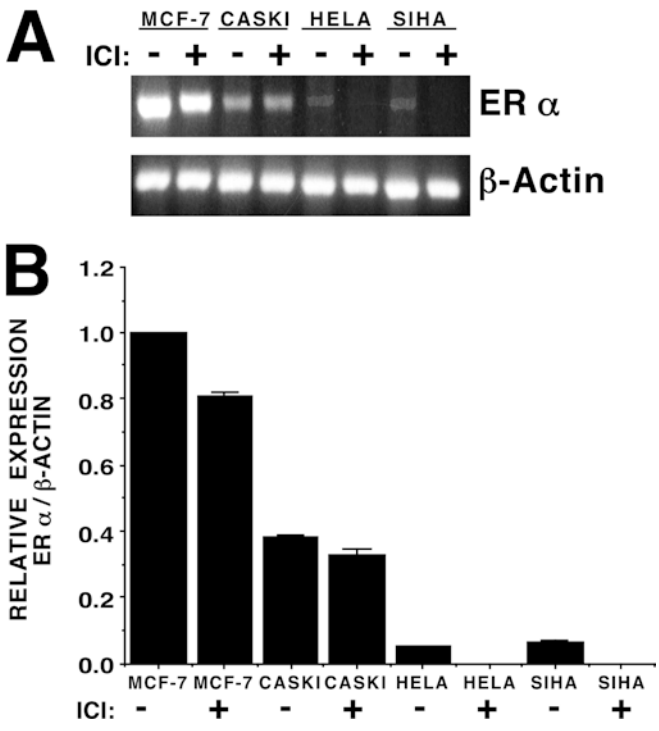


Fig. 3 **A** RT-PCR analysis of ER gene expression in control cells and ICI 182,780-pretreated cells. MCF-7 was used as a positive control and H_2O as negative control. Amplifying β-actin RT-PCR product provided a control for the amount of intact RNA used in the reaction. A representative result of three independent experiments is presented. **B** Semiquantitative analysis of the ratio ER gene expression to that of β-actin. The values are the means ± SEM of three independent experiments

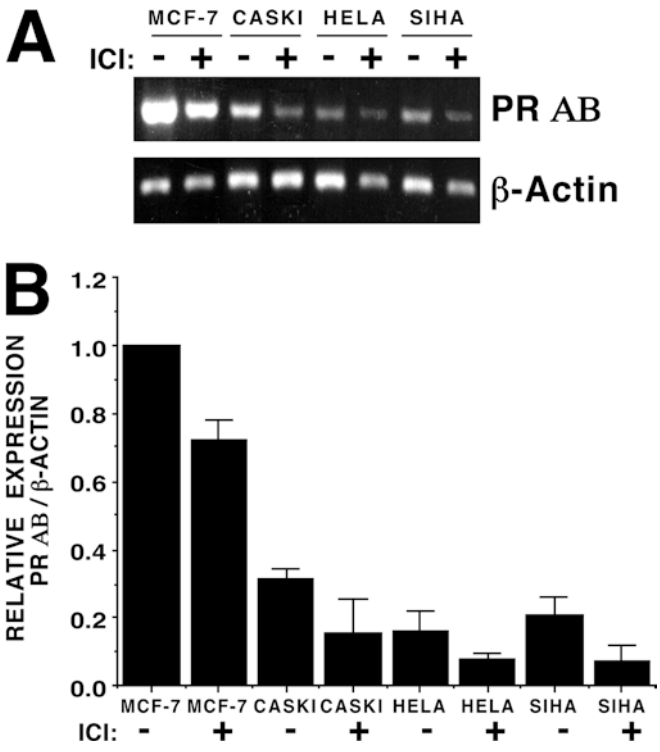


Fig. 4 **A** RT-PCR analysis of PR gene expression in control cells and ICI 182,780-pretreated cells. MCF-7 was used as a positive control and H_2O as negative control. Amplifying β-actin RT-PCR product provided a control for the amount of intact RNA used in the reaction. A representative result of three independent experiments is presented. **B** Semiquantitative analysis of the ratio PR gene expression to that of β-actin. The values are the means ± SEM of three independent experiments

Table 3 Percentage of cells in cell cycle phases treated with ICI 182,780 alone, cisplatin alone or the combination of cisplatin plus ICI 182,780. Values are the means ± SEM of three independent experiments for each point

Treatment	HeLa				SiHa				CaSki			
	G_0/G_1	S	G_2/M	G_2/G_1 ratio	G_0/G_1	S	G_2/M	G_2/G_1 ratio	G_0/G_1	S	G_2/M	G_2/G_1 ratio
Control	63 ± 1.5	24 ± 0.6	13 ± 0.9	20.6	68 ± 1.4	22 ± 1.8	11 ± 1.9	16.2	62 ± 1.4	20 ± 0.3	18 ± 1.0	29.0
ICI 182,780	53 ± 1.0	23 ± 0.9	25 ± 1.8*	47.1	62 ± 5.0	21 ± 0.5	17 ± 5.7	27.4	64 ± 2.7	17 ± 1.8	19 ± 1.2	29.7
Cisplatin	65 ± 2.6	33 ± 2.2*	1.0 ± 0.9*	1.5	67 ± 1.8	16 ± 1.3	17 ± 0.8	25.4	71 ± 4.9	13 ± 2.3	16 ± 2.7	23.0
ICI 182,780 + cisplatin	61 ± 1.3	8 ± 1.4*	32 ± 0.1*	52.5	68 ± 1.9	11 ± 4.4	21 ± 2.8	31.0	73 ± 6.0	13 ± 3.6	15 ± 3.0	20.0

*Significantly different from control by analysis of variance followed by Newman-Keuls test

expression, thus indicating that most cells may respond to estrogenic actions in the absence of its intracellular cognate receptors, and regardless of their origin [27]. Thus SiHa cells, considered to be squamous carcinoma cells, and CaSki cells that were derived from an epidermoid tumor metastatic to the small bowel mesentery, both showed chemosensitivity to cisplatin following exposure to ICI 182,780.

ICI 182,780, in addition to being a pure antiestrogen, has also been shown to have antiprogestin activity in PR^+ human breast cancer cell lines [32], as well to produce a significant loss in PR expression in tumors and in cell proliferation-related antigen, Ki67 [9]. These properties could offer additional benefits in drug combination.

In this study, we demonstrated that the combination of ICI 182,780 and cisplatin induced significant increase in G_2/M in cervical cancer cells except CaSki cells. Previous studies have also shown that doxorubicin combined with ICI 182,780 induces G_2/M blockage in MCF-7 breast cancer cells, confirming that this pure antiestrogen is more effective than tamoxifen in inducing G_2/M arrest [10]. Cervical cancer cells in the G_2/M phase of the cell cycle are believed to be more radiosensitive than those in the G_0/G_1 phase [18]. Additionally, radiotherapy has been demonstrated to be an effective

treatment for cervical carcinoma. Thus the concurrent use of cisplatin and radiotherapy with the possibility of interactions between radiation and chemotherapy, and the use of cisplatin in combination with ICI 182,780 could improve the treatment of cervical cancer.

CaSki cells had higher levels of ER gene expression than HeLa and SiHa cells. However, ICI 182,780 did not induce or downregulate this receptor, nor did it modify the cell cycle profile. Additionally, synergism in the cytotoxicity of these two drugs in combination which could not be explained by the mechanisms present in HeLa and SiHa cells deserves to be elucidated.

It is known that derivatives of platin (cisplatin and carboplatin) are fixed covalently to DNA-forming cisplatin-DNA adducts. This damage is eliminated by the nucleotide excision repair system of the DNA. It has also been shown that the high-mobility group (HMG) domain proteins such as HMG1 bind specifically to major cisplatin-DNA adducts and that the platinum-DNA-protein complex is involved in mediating cisplatin cytotoxicity by blocking nucleotide excision repair of the damaged DNA [21]. There is an inverse correlation between HMG1 and the ability of cells to repair damage both in vivo and in vitro. Recently, it has been reported that HMG1 is also able to facilitate the interaction of steroid hormone receptors such as ER and PR with their cognate DNA binding sites [16].

We are tempted to hypothesize, in relation to our results, that exposure to ICI 182,780 could lead to an increase in HMG1 expression and therefore to sensitization of ER^+ and PR^+ cells, such as CaSki cells, to treatment with cisplatin; this possibility deserves investigation. Previous studies have demonstrated that ICI 182,780 shows antiproliferative effects independent of ER, such as decreasing the insulin-like growth factor receptor (IGF-1) [5, 22], and decreasing transforming-growth factor-beta (TGF-beta) [25] or loss of C-fos expression [24]. Additionally, high intracellular concentrations (increasing or decreasing drug efflux), low metabolic inactivation, or an increase in other processes such as apoptosis, could be involved.

The pharmacological effects of ICI 182,780 have been evaluated in patients with advanced breast cancer. After treatment with ICI 182,780 at a monthly dose of 250 mg by intramuscular injection, the peak plasma concentration was approximately 13 ng/ml and side effects were minimal [19]. This level is lower than those seen in this study. However, there are reports that indicate a lack of serious adverse effects at higher doses both in experimental models [3, 17] and in women with primary breast cancer [9]. Therefore, it is possible to increase the doses administered and to reach higher serum levels.

Our findings suggest that this antiestrogenic compound could be a promising agent to be tested in combination with cisplatin not only in patients with cervical carcinoma, but also in those who are undergoing cisplatin therapy. Moreover, following our cell cycle data showing that progression can be stopped at the G_2/M checkpoint and the fact that failure of this control can lead to genomic instability resulting in hypersensitivity to radiation, it would be interesting to test whether the combination of ICI 182,780 and cisplatin used simultaneously with radiotherapy could increase the antiproliferative effect of the antineoplastic drug.

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