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IN VIVO MANIPULATION OF HUMAN BREAST CANCER GROWTH BY ESTROGENS AND GROWTH HORMONE: KINETIC AND CLINICAL RESULTS

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Summary—Since 1983, a series of experimental and clinical studies have been carried out on the possibility of enhancing the chemotherapy effectiveness in breast cancer by expanding the fraction of cycling cells. Theoretically estrogens should recruit breast cancer cells and this fact should result in a higher killing efficiency of antiproliferative drugs. Actually it has been clearly shown, by means of the thymidine labeling index and primer-dependent α-DNA polymerase assay, that low doses of diethylstilbesterol are able to increase the tumor proliferative activity of human breast cancer *in vivo* (estrogenic recruitment).

Three randomized trials have been carried out (one in locally advanced and two in metastatic breast cancer) comparing conventional polichemotherapy vs chemotherapy with estrogenic recruitment. Only limited advantages have been observed in these trials.

Searching for new modalities of kinetic manipulation of tumors, recombinant human growth hormone has been employed in a pilot study: the preliminary results indicate that it largely enhances tumor proliferative activity, suggesting the possibility of employing a growth factor system to increase chemosensitivity.

INTRODUCTION

Drug resistance is the major factor contributing to the failure of cancer chemotherapy. The Goldie and Coldman theory emphasizes the importance of the genetic origins of chemoresistance and suggests that, within a sensitive tumor population, a clone of drug-resistant cells could arise simply by chance mutation and/or amplification of a gene coding for one of the biochemical steps required for drug effectiveness. Thus, resistant cells are not induced by the toxic agent, but rather occur at a frequency dependent upon their spontaneous mutation rate; as the tumor cells population increases, the chances of mutation toward resistance increase, and the probability of cure decreases [1]. This model has provided the theoretical basis for a treatment strategy based on the rotation of non-cross-resistant combination regimens [2] and an early institution of chemotherapy, when the tumor burden is small [3].

Even if the somatic mutation theory appears to be the most useful working hypothesis, the possibility of other non-genetic causes of chemoresistance still has to be considered.

Cell kinetics studies indicate that the size of non-dividing (G_0) tumor cells may influence drug effectiveness; the larger the number of actively proliferating cells (S, G_2, M), the greater the killing efficiency of a drug regimen [4]. Furthermore, the *in vitro* therapeutic index of cytotoxic drugs may be improved by tumor growth factors that increase the proliferative rate of tumor cells [5]. These observations have important bearing on the design of new approaches for cancer treatment. Human breast cancer, which has been shown to be responsive to hormones and autocrine and/or paracrine growth factors, represents an appropriate model to test the clinical relevance of chemotherapy preceeded by hormone treatment in an attempt to enhance tumor growth kinetics.

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Since 1983, we have carried out a series of experimental and clinical studies in advanced breast cancer to evaluate the tumor cell recruiting activity of recombinant human growth hormone (rh-GH), which has been shown to induce the synthesis of the autocrine growth regulator of human breast cancer insulin-like growth factor 1 (IGF-1), and the feasibility and efficacy of chemotherapy preceded by estrogenic recruitment with low-dose diethylstilbesterol (DES). We now report the results of our experimental studies of *in vivo* cell kinetic effects of rh-GH and DES in patients with advanced breast cancer. We also report the results of three randomized clinical trials (one in locally advanced and two in metastatic breast cancer) comparing conventional chemotherapy vs cytokinetic chemotherapy with DES.

MATERIALS AND METHODS

DES-FAC in locally advanced breast cancer: pilot study

During the 26-month interval between September 1983 and October 1985, 39 patients with locally advanced breast cancer were consecutively treated with 3 DES-FAC 21 days as primary chemotherapy, followed by locoregional treatment (radical mastectomy and/or radiation therapy) and 3 DES-FAC alternating with 3 DES-CMF 21 days (DES-FAC = diethylstilbesterol 1 mg/os on days 1–3; 5-FU 600 mg/sm + doxorubicin 50 mg/sm + cyclophosphamide 600 mg/sm on day 4; DES-CMF = DES on days 1–3; methotrexate 40 mg/sm + 5-FU + CTX on day 4).

The aims of the study were to obtain data on tumor cell kinetic behavior during DES-FAC chemotherapy and to evaluate the feasibility and toxicity of such a regimen. Patients were required to have locally advanced disease (T3–T4, N2–N3 or inflammatory carcinoma) without distant metastases; ECOG performance status (PS) $\leqslant 2$; no contra-indication for anthracycline-containing chemotherapy; no previous therapy.

Of the 39 patients, 23 agreed to receive serial tumor biopses to allow for tumor cell kinetic studies during treatment. All patients had a large primary tumor and the first surgical biopsy procured enough tumor tissue to also establish histological diagnosis and receptor status; subsequent biopsies were performed with tru-cut needles in at least three different areas of the remaining tumor to avoid local variations in proliferative activity. Breast cancer kinetics were evaluated at the following times: before any treatment (T_0); after 3 consecutive days of DES (T_1); 24 h after the first FAC (T_2); at mastectomy after 3 courses of induction chemotherapy (T_3).

Tumor proliferative activity was studied by the thymidine labeling index (TLI) and primer-dependent α-DNA polymerase assay (PDP-LI). TLI represents a measure of the percentage of tumor cells in the DNA synthetic (S) phase of the cell cycle, while PDP-LI gives an *in vitro* estimation of the growth fraction. These two kinetic techniques have been described in detail elsewhere [6].

rh-GH in advanced breast cancer

The *in vivo* biological effects of rh-GH have been studied in 12 women with advanced breast cancer (locally advanced = 5 patients; metastatic = 7 patients; ER+ = 3 patients; ER− = 3 patients; unknown ER = 6 patients).

The following parameters were evaluated immediately before and 24 h after rh-GH (4 UI/day for 2 days): tumor TLI, tumor IGF-1 content, peripheral serum IGF-1 concentration.

Tumor and serum IGF-1 were measured by methods described elsewhere [7].

FAC vs DES-FAC in locally advanced breast cancer

A multicenter, randomized trial involving women with locally advanced breast cancer was started in November 1985.

Patients were randomized to receive 3 FAC ± DES 21 days followed by locoregional treatment and 3 FAC ± DES alternating with 3-CMF ± DES 21 days. Eligibility criteria included age $\leqslant 65$ yr, ECOG PS $\leqslant 2$, Stage IIIa–IIIb according to UICC-AJC classification and no previous hormonal therapy or chemotherapy. To date, 93 patients have entered this ongoing trial. Patients' characteristics were comparable in the two treatment arms.

CEF vs DES-CEF I in metastatic breast cancer

Between December 1983 and October 1985, 117 patients with metastatic breast cancer were randomly assigned to treatment with either conventional chemotherapy (CEF = cyclophosphamide 600 mg/sm + epi-doxorubicin 60 mg/sm + 5-FU 600 mg/sm on day 1, 21 days) or cytokinetic chemotherapy (DES-CEF = cyclophosphamide 600 mg/sm on day 1; diethylstilbesterol 1 mg/os on days 5–7; epi-doxorubicin

60 mg/sm + 5-FU 600 mg/sm on day 8, 21 days) for 11 cycles or until progression. Eligibility criteria were measurable metastatic disease, age ≤70 yr, ECOG PS ≤3, no involvement of the CNS and no previous chemotherapy for the metastatic situation; previous adjuvant polychemotherapy was admitted if terminated at least 6 months before relapse. The distribution of the patients' characteristics and the tumor characteristics was similar in the two treatment groups.

CEF vs DES-CEF II in metastatic breast cancer

In November 1985, a new trial was started in metastatic breast cancer; patients were randomized to receive CEF, with the same scheduling as the previous study, or DES-CEF II (diethylstilbesterol 1 mg/os on days 1–3; cyclophosphamide 600 mg/sm + epi-doxorubicin 60 mg/sm + 5-FU 600 mg/sm on day 4; 21 days) for 11 cycles or until progression.

The new scheduling for DES-CEF II was designed on the basis of the toxicity data recorded in DES-CEF I. This study was closed for accrual on January 1990 when 258 patients had been enrolled.

RESULTS

Experimental Results

Tumor cell kinetics during DES-FAC in locally advanced breast cancer

Table 1 shows the kinetic data obtained on 30 patients with locally advanced breast cancer (23 patients from the pilot study; 7 patients from the FAC vs DES-FAC randomized study) during DES-FAC chemotherapy.

A significant increase after DES was reported for TLI in 63.3% and for PDP-LI in 65.5% of the patients. At mastectomy (3–4 weeks after the third cycle of DES-FAC) the median TLI and the median PDP-LI were 38.8 and 54.7% of the basal value, respectively. Estrogenic recruitment was more frequently achieved in slowly rather than highly proliferating tumors: 73% of cases with low TLI (≤1.8%) vs 53% of cases with high TLI (>1.8%) and 80% of cases with low PDP-LI (≤4.2%) vs 50% of cases with high TLI (>4.2%) were recruited. Estrogenic recruitment was not related to ER status; a significant increase in TLI and PDP-LI was evident in 7/13 (54%) of ER+ and in 11/16 (69%) of ER− tumors.

Table 1. Tumor proliferative activity in locally advanced breast cancer during DES-FAC chemotherapy

Sample	TLI (%) (median)	PDP-LI (%) (median)
T_0	1.8 (0.2–19.4)	4.2 (0.8–22.5)
T_1	2.2 (0.2–18.5)	7.1 (0.4–24.8)
T_2	1.0 (0.2–23.5)	2.6 (0.9–9.5)
T_3	0.7 (0.1–6.2)	2.2 (0.4–13.7)

TLI = thymidine labeling index; PDP-LI = primer dependent α-DNA polymerase assay; T_0 = before any treatment; T_1 = after DES; T_2 = after the first cycle of chemotherapy; T_3 = at mastectomy.

Table 2. *In vivo* cytokinetic effects of rh-GH

Patient No.	ER status	TLI (%) basal	TLI (%) after rh-GH	Ratio
1	+	0.8	0.7	0.9
2	+	8.1	11.5	1.4
3	+	0.7	5.0	7.1
4	−	0.5	3.1	6.0
5	−	0.1	0.5	5.0
6	−	0.2	0.9	4.5
7	NA	1.2	0.2	0.1
8	NA	0.7	0.2	0.3
9	NA	0.5	0.5	1.0
10	NA	1.0	2.8	2.8
11	NA	1.1	3.5	3.2
12	NA	0.4	2.4	6.0

ER = estrogen receptor; TLI = thymidine labeling index; rh-GH = recombinant human growth hormone; NA = not available.

In vivo cell kinetic effects of rh-GH in advanced breast cancer

Before rh-GH administration, median tumor and serum IGF-1 levels were 4.64 ng/g (range 0.82–5.74 ng/g) and 63.5 ng/ml (range 38–197 ng/ml), respectively. After rh-GH, median tumor IGF-1 was 1.8 ng/g (range 0.84–4.33 ng/g) and median serum IGF-1 was 112 ng/ml (range 49–133 ng/ml).

Tumor TLI values before and after rh-GH are illustrated in Table 2; after rh-GH, an at least 2-fold increase in the basal value of TLI was recorded in 58.3% of cases.

Clinical Results

Locally advanced breast cancer: pilot study

Clinical results of our first study on DES-FAC chemotherapy have been reported in detail elsewhere [8]. The objective response rate to induction chemotherapy was 71.8% with 6 (15.4%) patients achieving complete response (CR) and 22 (56.4%) achieving partial response (PR). Non-responders comprised 28.2% of the patients: 7 minor response (MR), 3 stable disease (SD) and 1 progression (P). After induction chemotherapy and locoregional treatment 36/39 (92.3%) patients were disease-free.

To date, there have been 20 relapses and 17 deaths. The first site of relapse was locoregional

in 70%, distant in 25% and locoregional + distant in 5% of cases. Six years actuarial survival and progression-free survival are 51.7 and 48%, respectively.

Locally advanced breast cancer: randomized study FAC vs DES-FAC

At 4 yr from starting this adjuvant trial, the objective response rates to induction chemotherapy are: CR 4.6%, PR 55.8%, SD 37.2% and P 2.4% for the FAC arm; and CR 5.3%, PR 57.9% and SD 36.8% with no progressions for the DES-FAC arm; median survival is not reached for both the FAC and DES-FAC arms; median progression-free survival is 20 months for FAC and 22 months for DES-FAC.

Metastatic breast cancer: randomized studies

(a) CEF vs DES-CEF I. The results of this study have been published previously in detail [9]. Overall response rates (CR + PR) were similar in the two arms: 57.2% for CEF and 53.7% for DES-CEF, with slightly more patients in the DES-CEF arm achieving CR (24.1 vs 16.1%; P = NS). A significant advantage in the CR rate was observed in the DES-CEF arm for patients with soft tissue metastases (48 vs 27.7%, $P < 0.05$) and for patients with negative hormone receptors (35.7 vs 11.1%, $P < 0.025$).

At 6-yr follow-up, the median survival is 15 months for CEF and 22 months for DES-CEF; median progression-free survival is 10 months for CEF and 11 months for DES-CEF patients. These differences are not significant.

Survival rates were also analyzed by treatment according to previous adjuvant therapy. In patients previously treated with adjuvant chemotherapy, DES-CEF induced a significantly longer median survival (CEF 12 months, DES-CEF >27 months, $P = 0.029$) and progression-free survival (CEF 6 months, DES-CEF 8 months, $P = 0.04$) than CEF.

The DES-CEF regimen was more myelotoxic than conventional CEF: 77.2% of patients experienced at least one episode of leukopenia on the day of recycle vs 42.4% of patients in the CEF arm. Consequently, 43.3% of DES-CEF cycles had to be delayed in comparison with 11.8% of CEF cycles ($P < 0.0001$).

(b) CEF vs DES-CEF II. Overall response rates were similar in the two groups (47.3% for the CEF group and 50.9% for the DES-CEF group). The median survival was 17 months in the CEF arm and 20 months in the DES-CEF arm (P = NS). The median times to progression were 9 and 11 months for patients treated with CEF and DES-CEF, respectively ($P = 0.07$). The subgroup of patients not previously exposed to adjuvant chemotherapy showed a trend for a better median survival and a significantly better median progression-free survival when treated with DES-CEF (median survival: CEF 18 months vs DES-CEF 30 months, $P = 0.07$; median progression-free survival: CEF 8 months vs DES-CEF 14 months, $P = 0.001$). Once again, DES-CEF II was more myelotoxic than conventional CEF; 52.4% of DES-CEF patients experienced at least grade 1 leukopenia in comparison to 31.7% of CEF patients ($P = 0.007$).

DISCUSSION

Our experimental data obtained in locally advanced breast cancer confirm, in a larger series of patients than previously reported [6, 8], that *in vivo* administration of low-dose DES induces an expansion of breast cancer cell growth, and that subsequent inhibition of tumor cell proliferation can be achieved by chemotherapy at the time of maximally induced cell recruitment.

Among the clinical and biological parameters, only the basal proliferative activity was effective in predicting tumor cell recruitment after DES: slowly proliferating tumors (below median TLI and/or PDP-LI) were more frequently stimulated by DES than highly proliferating tumors.

Interestingly, DES recruitment occurred in 54% of ER+ and in 69% of ER− tumors. Therefore, the presence of hormonal receptors is not an essential prerequisite to achieve an estrogenic recruitment. Similarly, *in vitro* studies [10] have shown that physiological doses of estrogen increased the TLI in human breast cancer even in the absence of ERs. A possible explanation for this observation is based on the presence in all ER− tumors of some ER+ cells that, following DES, could produce mitogenic growth factors that affect the ER− component [11].

Clinical results of our first pilot study on locally advanced breast cancer were promising: 72% of patients achieved an objective response after 3 cycles of DES-FAC and 92% were rendered disease-free on completion of induction chemotherapy and locoregional treatment. At 6 yr from starting the study the median survival was not reached. It is important to stress the fact that all the patients enrolled had unfavourable

clinical signs at presentation (i.e. large tumor masses, satellite skin nodules or ulceration, fixation or axillary lymph nodes and/or involvement of supraclavicular lymph nodes) and 14/39 (36%) of patients had inflammatory breast carcinoma. The early results of this study prompted us to test DES chemotherapy in prospectively randomized trials.

In the first study on metastatic breast cancer (CEF vs DES I), two completely different chemotherapy schedules were used; the scheduling of DES-CEF I was designed to exploit the kinetic recruitment induced by cyclophosphamide (as demonstrated) in ovarian cancer and myeloma [12, 13]) and DES. This study showed that chemotherapy with estrogenic stimulation can offer statistically, an advantage for certain subsets of patients, but no survival advantage favoring the whole group. Furthermore, the cytokinetic arm was significantly more myelotoxic than conventional arm. This toxicity was attributed to the different scheduling used for chemotherapy. The second trial on metastatic breast cancer (DES-CEF II), showed no survival benefit with a trend for a better relapse-free survival for DES-CEF. Cytokinetic chemotherapy was again more myelotoxic than CEF, although in the two treatment arms all chemotherapeutic drugs were administered on the same day. This finding suggests that DES itself induces a more pronounced bone marow chemosensivity, possibly through a direct kinetic effect on progenitors cells.

A retrospective analysis of the two randomized trials, including 375 patients with metastatic breast cancer, showed a clear advantage in median survival (18 vs 12 months, $P = 0.01$) and progression-free survival (12 vs 7 months, $P = 0.006$) for the patients with ER− tumors receiving DES-CEF. This clinical finding is in keeping with our experimental data showing that ER− tumors are more prone than ER+ tumors to respond to 3 days of DES stimulation. However, this clinical result should be interpreted with caution since the ER status was not known in the entire patient population.

A flare phenomenon was recorded in 4% of the cases after the first day of administration of DES. All the patients with flare had ER+ tumors and we may infer that estrogen recruitment could be achieved more promptly in this subset of tumors.

In conclusion, our results indicate that breast cancer cells can be stimulated *in vivo* with DES, regardless of the prevalent ER status of the tumor; chemotherapy with estrogenic recruitment is feasible and, in spite of the expected chemoresistance of metastatic breast cancer, can produce some favorable results in this stage of disease. Appropriate studies should be designed to better define the optimal dose and scheduling of DES for each subgroup of patients (i.e. according to ER status) and to explore the validity of this investigational approach at an early stage of disease where kinetic and genetic chemoresistance are less likely to occur.

Furthermore, our preliminary results have shown that *in vivo* administration of rh-GH can significantly increase tumor proliferative activity in advanced breast cancer.

These results are encouraging and indicate that more potent and selective recruiting agents should be tested to develop more effective chemotherapy protocols.

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