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Estradiol and Progesterone Receptors Content and Response to Norethisterone Treatment in Advanced Breast Cancer*

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Abstract—*Twenty-six patients with advanced breast cancer and progressive disease were treated by norethisterone. Estradiol and progesterone receptors were measured before starting treatment, and in three cases after 2 to 4.75 months of therapy. Partial remissions (greater than 50% decrease of all lesions) or objective improvements (decrease of 20–50%) were obtained in 7 patients, with a mean duration of 6.7 months. Stabilization of the lesions (no change or decrease of less than 20% in the size of the target lesion) was observed in 7 patients. No clear-cut correlation was found between clinical response and presence in biopsies of estradiol and progesterone receptors. However, absence of response occurred more frequently if tumors did not contain progesterone receptors.*

INTRODUCTION

PROGESTIN therapy has been shown to induce objective tumor regression in some cases of metastatic breast cancer [1, 2]. Two types of compounds have been used: 17-hydroxyprogesterone derivatives (medroxyprogesterone and its acetate, megestrol [3–10]) and 19-nortestosterone derivatives (norethisterone and its acetate [11–16]). Results of clinical tests give a wide range of response rates: 3.6–41% for norethisterone and 0–36% for 17-hydroxyprogesterone derivatives.

These compounds are very well tolerated, have few side effects and may succeed after failure of prior endocrine therapy or chemotherapy. The usefulness of progestins would be greatly increased if it were possible to predict which patients would respond to such treatment. A plausible hypothesis was that only tumors exhibiting progesterone receptors regressed under progestagen treatment. An investigation testing this hypothesis is described here.

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MATERIALS

Patients

Twenty-six patients (25 women, 1 man) with advanced breast cancer and evidence of progressive disease were treated with a 80-mg daily dose of norethisterone, as recommended by the manufacturer, administered orally (except patient 2, who received 50 mg daily). The patients (Table 1) showed either locally advanced inoperable tumors or disseminated cancers, or progressive disease after prior endocrine therapy (castration, androgens, or tamoxifen), radiotherapy or chemotherapy. All had accessible lesions to be biopsied for receptor measurements.

Before beginning the treatment by norethisterone (17α-ethinyl-19-nortestosterone), the patients were evaluated by chest X-ray examination, liver or bone scintigraphy and measurement of the lesions chosen as the target for evaluation of the effectiveness of the treatment. We excluded from the study patients displaying non-evoluting lesions during the preceding 2 months, patients previously treated by progestagens and patients treated by endocrine therapy or chemotherapy less than one month before entering the trial. Tumor biopsies were taken before beginning the treatment, and a minimum of 2 months of treatment by norethisterone was required for the first evaluation of the results.

Table 1. *Clinical characteristics of the patients*

Patient No.	Age (yr)	Hormonal status	TNM stage at first diagnosis	Previous treatment for advanced disease*	Dominant site used for assessment
1	72	Menopaused	II	0	Skin metastases and controlateral breast
2	64	Menopaused	III	Chemotherapy	Skin metastases and controlateral breast
3	73	Menopaused	II	0	Pleural metastases
4	72	Menopaused	III	Chemotherapy	Skin metastases
5	68	Castrated (40 yr)	II	0	Skin metastases
6	66	Menopaused	n.d.†	Chemotherapy	Skin metastases
7	56	Menopaused	n.d.†	0	Skin metastases
8	60	Menopaused	III	Chemotherapy	Upper clavicular lymph node
9	52	Castrated (49 yr)	II	Chemotherapy	Skin metastases
10	41	Normal cycle	III	0	Skin and lung metastases
11	72	Menopaused	n.d.†	Chemotherapy	Skin and pleural metastases
12	62	Menopaused	III	Chemotherapy	Skin metastases
13	74	Menopaused	III	Chemotherapy	Skin metastases
14	60	Menopaused	n.d.†	Chemotherapy	Skin metastases
15	66	Menopaused	III	Chemotherapy	Lung metastases
16	60	Menopaused	III	0	Lymph nodes, liver, skin and pleural metastases
17	55	Castrated (52 yr)	III	Chemotherapy	Skin metastases
18	47	Castrated (44 yr)	III	Tamoxifen Chemotherapy	Skin metastases
19	57	Menopaused	n.d.†	Chemotherapy Hormonotherapy	Skin metastases Lymph node metastases
20	51	Castrated (46 yr)	n.d.†	0	Lymph nodes and skin metastases
21	72	Menopaused	n.d.†	0	Skin metastases (local recurrence)
22	42	n.d.	n.d.†	Chemotherapy	Skin metastases
23	65	Menopaused	III	0	Skin metastases and controlateral breast
24	53	Castrated	II	Chemotherapy	Lung metastases
25	66	Menopaused	III	Chemotherapy androgen + tamoxifen	Skin metastases
26	78	Male patient	n.d.†	0	Skin metastases

*All patients received previous radiotherapy except patients Nos 5, 6, 7, 11, 15, 19, 20, 21 and 26.
†n.d.: not determined or not known; first diagnosis outside institution.

Then each patient was examined every 6–8 weeks, including clinical and radiological controls similar to the first evaluation. For obvious ethical reasons, 2 patients showing evidence of progressive disease during the initial phases of progestin therapy received other treatments and were not taken into account in this study. Two other patients died during the first month of treatment, and one patient was excluded because of side effects of the treatment.

Norethisterone therapy was continued until there was evidence of failure to respond or until relapse following remission.

Methods

Response quantification was based on the product of the 2 perpendicular diameters of the target lesion.

Criteria used for evaluating the response to treatment were those of the EORTC Clinical Screening Cooperative Group [17–19]:

- E_4: disappearance of all known lesions during at least one month (complete remission);
- E_3: greater than 50% decrease of known lesions, and no new lesions appearing, for a minimum of one month (partial remission);
- E_2: regression of less than 50% and more than 20% of measurable lesions, for a minimum of one month;
- E_1: no change, or less than 20% decrease in the size of measurable lesions;
- E_0: progression of some or all lesions, or appearance of new lesions.

In the case of dissociated responses from several lesions, only the weakest response was taken into account and was classified E_0 if any target lesion progressed during the trial period, even if other lesions regressed. Duration of response was dated from the start of therapy until either new lesions appeared or existing lesions increased by 25% or more.

Three patients (Nos 1, 10, 23) underwent a second biopsy after treatment, performed three days after withdrawal from norethisterone.

Estradiol receptors were measured using tritiated estradiol (sp. act. ~ 100 Ci/mmol) as ligand and excess of non-radioactive diethylstilbestrol for estimation of non-specific binding. The assay protocol was that of EORTC [20]. Progesterone receptors were measured as previously described [21] with tritiated progesterone in the presence of non-radioactive cortisol as ligand.

The physicians who evaluated the patients were unaware of the results of receptor assays.

RESULTS

Responses to treatment

Table 2 summarizes the results of receptor measurements and responses to norethisterone therapy.

Three patients (12%) achieved partial regression (E_3). Objective improvement (E_2) was observed in 4 patients (15%), and no response or less than 20% decrease in the size of the target lesion (E_1) was shown by 7 (27%) patients. Mean duration of E_3 and E_2 responses was 6.7 ± 1.1 (S.D.) months.

Correlation between receptor concentration and clinical response

Estradiol receptor (ER) was present in appreciable amounts (≥ 10 fmol/mg protein) in 88.5% of the tumors, and progesterone recep-

Table 2. ER and PGR concentration in the biopsies and responses to norethisterone

Patient No.	Months of treatment	Response	Duration of response (months)	ER concentration (fmol/mg protein)	PgR concentration (fmol/mg protein)
1	10.50*	E_3	9*	9†	24†
				0†	64‡
2	7.25	E_3	7	190	106
3	6.00*	E_3	6*	147	0
4	7.75	E_2	7	16	12
5	6.25	E_2	6	318	0
6	6.00	E_2	6	192	17
7	6.25	E_2	6	17	132
8	6.75	E_1	6	132	0
9	6.25	E_1	6	18	0
10	5.25	E_1	5	292†	394†
				0§	0§
11	4.00	E_1	4	130	17
12	4.50	E_1	4	122	126
13	4.25	E_1	4	10	20
14	3.75	E_1	3	135	0
15	4.00	E_0	—	762	0
16	2.50	E_0	—	428‖	0‖
				682¶	0¶
17	1.00**	E_0	—	382	0
18	2.00	E_0	—	259	285
19	4.00	E_0	—	256	0
20	2.5	E_0	—	174	12
21	4.00	E_0	—	85	0
22	1.75**	E_0	—	49	23
23	2.25	E_0	—	31†	29†
				42††	13††
24	2.25	E_0	—	0	9
25	2.00	E_0	—	0	0
26	2.00	E_0	—	234	0

*Continued; †concentration before treatment; ‡concentration after 2 months of treatment; §concentration after 4.75 months of treatment; ‖receptors assays were performed on skin metastases; ¶receptors assays were performed on lymph node metastases; **patient included in the study because of evidence of response before 2 months; ††concentration after 2.25 months of treatment.

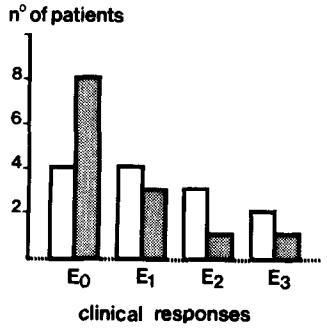

Fig. 1. Responses to norethisterone treatment and progesterone receptor status. Shaded bars = progesterone receptor-negative tumors. White bars = progesterone receptor-positive tumors. The difference between E_0 and E_3 is significant (P = 0.05).

tor (PgR) in 50.0% of the tumors before treatment. The mean concentration of ER or PgR of responders (E_1, E_2 and E_3) and non-responders (E_0) does not differ significantly ($P > 0.05$), and analysis of variance for PgR distribution within the 4 categories of response gives an F-value of 2.65 ($P > 0.05$).

However, absence of response is more likely to be observed among PgR-negative tumors (Fig. 1). The proportion of non-responding patients (E_0) (8/13) is significantly different from the patients displaying partial remission (E_3) (1/13) among PgR-negative tumors ($P = 0.05$).

The effect of norethisterone administration on ER and PgR concentration in metastases

This could be observed in 3 patients. Patient 10 was premenopausal and showed high concentrations of both ER and PgR before treatment (biopsy taken at day 26 of cycle). Both receptors had disappeared after 4.75 months of treatment (biopsy taken at day 6 of cycle). No significant variation in receptor concentration was observed for patient 23 (2.25 months of treatment). Patient 1 showed partial remission after 2 months of treatment, with an increased concentration of PgR. However, because of the very small number of cases, no conclusions could be drawn regarding the change of receptor status possibly induced by norethisterone therapy.

DISCUSSION

Compared to the other forms of hormonal therapy used in late breast cancer, the use of progestagens has remained limited. Oral progesterone has been found inactive by Volk *et al.* [22]. 17-Hydroxyprogesterone caproate combined with estrogens gave 27% objective remissions in 22 postmenopausal patients with advanced breast cancer previously resistant to estrogen therapy alone [3].

Medroxyprogesterone and its acetate have been studied recently by several groups using a high dose (500–1500 mg/day) [4–8]. In an early investigation by Stoll [2], 200–400 mg daily produced 2/12 (17%) objective responses. Rates of response (including complete and partial remissions) to high-dose medroxyprogesterone acetate ranged from 28 to 48% [4–8]. Megestrol, another 17-hydroxyprogesterone derivative, gave a 23% response rate (Ansfield *et al.* [23]) and 50% responses in a recent report of Teulings *et al.* [9], with 150–180 mg/day in 34 postmenopausal patients.

The first investigation of norethisterone reported was by Lewin *et al.* [11]. These authors obtained 19% objective regressions in 26 castrated or postmenopausal patients receiving 40 mg/day of norethisterone. Response rates with norethisterone acetate varied between 17% (Stoll, 60 mg/day [2]) and 50% [12, 13].

In our study we obtained 12% objective partial remissions and 15% objective improvements with 80 mg/day norethisterone (patient 2 received 50 mg/day). This is a relatively low percentage of positive responses. The selection of patients, including a majority of patients resistant to prior chemotherapy and/or hormonotherapy and displaying advanced disease, may account for the low rate of responses. Moreover, the standardized criteria used for assessment of therapeutic response in breast cancer are probably more strict than those used in the early studies.

It is now well-established that ER and PgR determinations are useful guides for selecting patients able to respond to endocrine therapy [24]. From our study no correlation could be drawn between clinical response and presence or concentration of ER and PgR in the metastases before treatment by norethisterone. This lack of correlation may be due to the fact that at high doses some progestins may act through another steroid receptor. For example, the results of Teulings *et al.* [9] show a correlation between tumor regression by megestrol treatment and the presence of large amounts of androgen receptors in the tumors. In turn, a single determination of receptor levels could be insufficient. Progesterone receptor is under complex hormonal control involving estrogen and progesterone action. Its concentration thus probably varies under the effect of hormonal treatment. During the preparation of this manuscript a study was published on estradiol, androgen, glucocorticoid and progesterone receptors in progestin-induced regression of human breast cancer [9], where a lack of correlation between estradiol

and progesterone receptors and effect of treatment by megestrol acetate was also noticed.

In conclusion, it does not seem possible to increase the proportion of patients responsive to progestin therapy through selection based on progesterone receptor presence in the tumor. However, when progesterone receptor is not detected in the tumor, absence of therapeutic response is more probable.

REFERENCES

1. KENNEDY BJ. Hormone therapy for advanced breast cancer. *Cancer* 1965, **18**, 1551–1557.
2. STOLL BA. Progestin therapy of breast cancer: comparison of agents. *Br Med J* 1967, **3**, 338–341.
3. CROWLEY LG, MACDONALD I. Delalutin and estrogens for the treatment of advanced mammary carcinoma in the postmenopausal woman. *Cancer* 1965, **18**, 436–446.
4. PANNUTI F. Moderne prospettive nel trattamento del cancro della mammella e delle sue metastasi. *Minerva Chir* 1977, **32**, 1211–1220.
5. MATTSSON W. High dose medroxyprogesterone-acetate treatment in advanced mammary carcinoma. A phase II investigation. *Acta Radiol Oncol Radiat Phys Biol* 1978, **17**, 387–400.
6. PANNUTI F, MARTONI A, LENAZ GR, PIANA E, NANNI P. A possible new approach to the treatment of metastatic breast cancer: massive doses of medroxyprogesterone acetate. *Cancer Treat Rep* 1978, **62**, 499–504.
7. ROBUSTELLI DELLA CUNA G, CALCIATI A, BERNARDO STRADA MR, BUMMA C, CAMPIO L. High dose medroxyprogesterone acetate (MPA) treatment in metastatic carcinoma of the breast: a dose-response evaluation. *Tumori* 1978, **64**, 143–149.
8. DE LENA M, BRAMBILLA C, VALAGUSSA P, BONADONNA G. High-dose medroxyprogesterone acetate in breast cancer resistant to endocrine and cytotoxic therapy. *Cancer Chemother Pharmacol* 1979, **2**, 175–180.
9. TEULINGS FAG, VAN GILSE HA, ENKELMAN MS, PORTENGEN H, ALEXIEVA-FIGUSH J. Estrogen, androgen, glucocorticoid and progesterone receptors in progestin-induced regression of human breast cancer. *Cancer Res* 1980, **40**, 2557–2561.
10. ALEXIEVA-FIGUSH J, VAN GILSE HA, HOP J, PHOA CH, BLONK-WIJST J, TREURNIET RE. Progestin therapy in advanced breast cancer: megestrol acetate—an evaluation of 160 treated cases. *Cancer* 1980, **46**, 2369–2372.
11. LEWIN I, SPENCER H, HERMANN J. Clinical and metabolic effects of 17α-ethinyl-19-nortestosterone in mammary carcinoma. (Abstr.) *Proc Am Assoc Cancer Res* 1959, **3**, 37.
12. CURWEN S. The value of norethisterone acetate in the treatment of advanced carcinoma of the breast. *Clin Radiol* 1963, **14**, 445–446.
13. CLAVEL B, BOURDIN JS. Les progestatifs dans le traitement du cancer du sein à une phase avancée. *Sem Hop Paris* 1970, **46**, 170–188.
14. EDELSTYN GA. Norethisterone acetate (SH_{420}) in advanced breast cancer. *Cancer* 1973, **32**, 1317–1320.
15. RUBENS RD, KNIGHT RK, HAYWARD JL. Norethisterone acetate in the treatment of advanced breast cancer. *Eur J Cancer* 1976, **12**, 563–565.
16. RUBENS RD, BEGENT RHJ, KNIGHT RK, SEXTON SA, HAYWARD JL. Combined cytotoxic and progestagen therapy for advanced breast cancer. *Cancer* 1978, **42**, 1680–1686.
17. CHAUVERGNE J, CLAVEL B, GARYBOBO J *et al.* Le recueil des informations dans le traitement médical des cancers. Utilisation d'un langage commun sur fiche normalisée. *Sem Hop Paris* 1974, **50**, 3119–3125.
18. CHAUVERGNE J, HOERNI B. Proposition pour une normalisation d'appréciation des résultats thérapeutiques. *Bordeaux Med* 1971, **4**, 1513–1514.
19. ORGANISATION MONDIALE DE LA SANTÉ. Manuel de l'OMS: notification des résultats du traitement du cancer. OMS publication offset No. 48. Genève, OMS, 1980, 23–42.
20. EORTC BREAST CANCER COOPERATIVE GROUP. Standard for the assessment of estrogen receptors in human breast cancer. *Eur J Cancer* 1973, **9**, 379–381.
21. PICHON MF, MILGROM E. Characterization and assay of progesterone receptor in human mammary carcinoma. *Cancer Res* 1977, **37**, 464–471.

22. VOLK H, ESCHER GC, HUSEBY RA, TYLER F, CHEDA J. Hormonal therapy in carcinoma of the breast. I. Effect of oral progesterone on clinical course and metabolism of nitrogen and selected electrolytes and steroids. *Cancer* 1960, **13**, 757–763.
23. ANSFIELD FJ, DAVIS HL, ELLEBY RA, RAMIREZ GA. Clinical trial of megestrol acetate in advanced breast cancer. *Cancer* 1974, **33**, 907–910.
24. MCGUIRE WL. Steroid hormone receptors in breast cancer treatment strategy. *Recent Prog Horm Res* 1980, **36**, 135–156.