

Increase of Progesterone Receptor by Tamoxifen as a Hormonal Challenge Test in Breast Cancer¹

Moise Namer, Claude Lalanne, and Etienne-Emile Baulieu²

Centre Anticancéreux A. Lacassagne, 06054 Nice [M. N., C. L.] and Inserm U 33, Faculté de Médecine de Bicêtre, Université de Paris Sud, 94270 Bicêtre [E-E. B.] France

ABSTRACT

In 25 cases of postmenopausal breast cancer, estradiol receptor (ER) and progesterone receptor (PR) were measured in cutaneous metastatic nodules before and after administration of 30 mg of tamoxifen per day for 1 week. No response was recorded in ER-poor cases. However, in tumors containing >10 fmol ER per mg cytosol protein, 6 of 14 cases showed an increase in PR of >30 fmol/mg cytosol protein. The presence or absence of PR before administration of tamoxifen did not discriminate systematically between hormone-responsive and nonresponsive tumors. These findings demonstrate *in vivo* that biochemical changes brought about by an agent binding to ER can be observed only in ER-positive cases. In addition they suggest that, in these ER-positive cases responding to tamoxifen by increase of PR, the simultaneous or sequential administration of both antiestrogen (rescuing PR) and progestagen (decreasing PR) may allow better hormonal control of the disease.

Sixty to 70% of postmenopausal breast cancers are ER³ "positive". Of those, about 50% respond to hormonal manipulation, whether it be ablative surgery or administration of androgen, estrogen, progestagen, or, more recently, antiestrogen (11, 15, 16, 19).

Progestagens share with the naturally occurring progesterone the property to counteract the growth-promoting effects of estradiol on several target tissues. However, they have been one of the least used hormonal adjuvant treatments in breast cancers (13, 20, 22, 23, 25), even if the "extinction" effect of estradiol plus progesterone administration on experimental mammary tumors induced by 7,12-dimethylbenzanthracene has been previously observed (10). Approximately 50% of postmenopausal cancers have measurable concentrations of PR, of which >90% belong to the ER-positive group (1, 6, 16). Moreover, it has been established in the guinea pig uterus (18) that progesterone administration tends to decrease PR binding site concentration. Regardless of the mechanisms involved, this constitutes short-feedback down-regulation system by agonistic compounds, limiting their activity. On the contrary, as originally demonstrated in the uterus (18) and confirmed in

human cancer cells in culture (7), estrogens increase the PR concentration.

Tamoxifen is a nonsteroidal antiestrogen which binds to the ER and has already been used satisfactorily in the therapy of ER-positive breast cancers (14, 19). In mammals, it displays both antiestrogenic effects and some estrogenic properties; in particular, it increases the PR concentration in the rodent uterus (12). Recently, a 40-mg/day for 5 to 7 days test was performed in postmenopausal endometrial carcinomas, resulting in increased PR, while ornithine decarboxylase activity, an index of cell growth, was not modified (21).

These considerations prompted us to measure ER and PR in cutaneous metastases in 25 cases of advanced breast cancer, before and after 30 mg of tamoxifen per day for 7 days. We hoped to investigate the possibility that tamoxifen, presumably harmless in terms of growth-promoting effect on the tumors, could help in obtaining appropriate PR concentrations potentially useful to mediate the beneficial effects expected from progestagens. In this communication, we report results concerning ER and PR measured with techniques determining the cytosol receptors readily labeled by radioactive hormonal ligands *in vitro* at 0 to 4° (15, 16) with the dextran-coated charcoal assay (3). Appropriate technical controls were performed according to the previously described principles (4), including receptor concentration measurements in neighboring nodules at 1-week intervals in 2 untreated patients (Table 1).

In all cases where ER was originally present (Table 2), it became virtually not detectable after administration of tamoxifen. This is probably due to transfer of the receptor into the nucleus (21) or to the antiestrogenic effect of tamoxifen (estrogens increase their own receptor concentration). Such a constant effect is in itself an indication that the target cells have been reached by the drug. While there was a significant rank correlation of the concentration of PR with that of ER in untreated tumors, the increase in PR after administration of tamoxifen was never observed in the 9 ER-poor cases (<10 fmol/mg cytosol protein). This latter finding confirms the predictive negative value of low ER concentrations, indicating that the tumors generally do not respond to hormonal therapy (6, 11), including tamoxifen (14, 19). However, of 15 tumors with >10 fmol ER per mg protein, 6 tumors showed a definitive increase in PR (>30 fmol/mg protein) after tamoxifen and 9 tumors did not respond positively (whether originally PR > 30 fmol/mg protein as in Cases 17 and 19 or = 0 as in Cases 10, 11, and 22). An additional negative case (Case 25) had a strong response, and, therefore, the original level of PR does not discriminate systematically between hormone-responsive and nonresponsive tumors (8). Consequently, as in endometrial

¹ Supported in part by Inserm Contract 76.5.478.

² To whom requests for reprints should be addressed, at Lab Hormones, Université de Paris Sud, 78 Avenue du Général Leclerc, 94270 Bicêtre, France.

³ The abbreviations used are: ER, estradiol receptor; PR, progesterone receptor.

Received November 20, 1979; accepted February 22, 1980.

Table 1

ER and PR in breast cancer cutaneous metastases at 1-week intervals
Patients did not ever receive any adjuvant therapy. The "first" and "second" biopsies were obtained from neighboring cutaneous nodules.

Patient age (yr)	fmol/mg cytosol protein			
	1st biopsy		2nd biopsy	
	ER	PR	ER	PR
66	23	9	22	4
70	28	4	26	5

Table 2

ER and PR in breast cancer cutaneous metastases before and after tamoxifen (30 mg for 7 days)

Patients did not ever receive any adjuvant therapy. The biopsies "before" and "after" were obtained from neighboring cutaneous nodules; occasionally 2 s.c. biopsies were performed on 2 nodules, and the same receptor concentrations were found.

Rank correlation coefficient according to the method of Spearman (24) was: between ER and PR before tamoxifen ($r_s = 0.68$), statistically significant ($p < 0.05$); between ER before tamoxifen and the increase of PR by tamoxifen (PR after - PR before) ($r_s = 0$), not statistically significant; and between PR before tamoxifen in cases where ER > 20 fmol/mg protein and the corresponding increase of PR ($r_s = 0$), not statistically significant. Calculations by Dominique Drouet.

Case	Patient age (yr)	fmol/mg cytosol protein			
		Before tamoxifen		After tamoxifen	
		ER	PR	ER	PR
1	71	0 ^a	0	0	0
2	85	0	0	6	5
3	60	0	0	0	0
4	54	0	30	ND	0
5	49	0	0	0	0
6	58	0	4	4	0
7	58	3	0	2	0
8	72	7	0	0	0
9	68	9	0	0	4
10	59	11	0	ND	0
11	71	13	0	0	11
12	72	20	7	5	13
13	72	25	20	ND	50
14	84	40	20	20	20
15	63	53	92	12	1120
16	70	80	110	17	980
17	80	85	60	7	50
18	69	92	34	11	605
19	77	120	105	0	0
20	57	120	1030	12	1600
21	65	185	190	25	440
22	57	225	0	8	0
23	70	350	65	9	205
24	67	430	ND	11	30
25	75	ND	0	25	770

^a 0, <1 fmol/mg cytosol protein; ND, not determined for technical reasons.

cancer (21), a dynamic test may be more valuable than a single set of receptor measurements.

These data, obtained with an agent binding to the ER, are the first to demonstrate directly in human breast cancer the necessity of this receptor to obtain a biochemical response. Although some of the results are difficult to interpret because of borderline values (Cases 8 to 13) or incomplete information (Cases 24 and 25), it appears that among ER-positive cases, some respond to tamoxifen and others do not. This finding may be comparable to the ~50% ER-positive tumors that are objectively influenced by hormonal therapy (6, 11, 15). These observations are preliminary, but it is proposed that the ER-positive tumors responding to tamoxifen by an increase of PR could subsequently be better controlled by progestagen ther-

apy. In such cases, it is conceivable that simultaneous or sequential administration of the antiestrogen can rescue the PR concentration, negatively influenced by progestagen, and that the combined treatment may become a very important mode in the hormonal management of breast cancer. We are presently studying doses and duration of treatments with the aim of increasing PR enough by tamoxifen in order to improve potentially progestagen action, without depressing ER by the progestagen itself, as may occur in view of experiments with the uterus (9, 17). In any case, it should be stressed that the situation is not simple, as also underlined by our recent findings of tamoxifen-progesterone synergistic activities in the chick oviduct system (5).

These results show that steroid receptors can be monitored pharmacologically (2) in breast cancer *in vivo* and suggest that a rational approach in the hormonal biology of tumors may have reached the stage of usefulness in the treatment of this disease.

ACKNOWLEDGMENTS

This paper is dedicated to our friend, Elwood V. Jensen, on his 60th birthday (January 13, 1980). We are happy that our data, while introducing a new approach to study the hormonal responsiveness of breast cancer, confirm his pioneering observations (11).

REFERENCES

- Allegra, J. C., Lippman, M. E., Thompson, E. B., Simon, R., Barlock, A., Green, L., Huff, K. K., Do, H. M. T., and Aitken, S. C. Distribution, frequency, and quantitative analysis of estrogen, progesterone, androgen, and glucocorticoid receptors in human breast cancer. *Cancer Res.*, 39: 1447-1454, 1979.
- Baulieu, E. E. Current approaches to steroid hormone-cell interactions. In: K. Fuxe, T. Hökfelt, and R. Luft (eds.), *Central Regulation of the Endocrine System*, pp. 239-260. New York: Plenum Press, 1979.
- Baulieu, E. E., Alberga, A., and Jung, I. Récepteurs hormonaux: liaison spécifique de l'oestradiol à des protéines utérines. *C. R. Hebd. Seances Acad. Sci.*, 265: 354-357, 1967.
- Bayard, F., Damilano, S., Robel, P., and Baulieu, E. E. Cytoplasmic and nuclear estradiol and progesterone receptors in human endometrium. *J. Clin. Endocrinol. Metab.*, 46: 635-648, 1978.
- Catelli, M. G., Binart, N., Elkik, F., and Baulieu, E. E. Effect of tamoxifen on oestradiol and progesterone induced synthesis of ovalbumin and conalbumin in chick oviduct. *Eur. J. Biochem.*, in press, 1980.
- De Sombre, E. R., Carbone, P. P., Jensen, E. V., McGuire, W. L., Wells, S. A., Wittliff, J. A., and Lipssett, W. B. Steroid receptors in breast cancer (Special Report). *N. Engl. J. Med.*, 301: 1011-1012, 1979.
- Horwitz, K. B., and McGuire, W. L. Estrogen control of progesterone receptor in human breast cancer: correlation with nuclear processing of estrogen receptor. *J. Biol. Chem.*, 253: 2223-2228, 1978.
- Horwitz, K. B., McGuire, W. L., Pearson, O. H., and Segaloff, A. Predicting response to endocrine therapy in human breast cancer: a hypothesis. *Science (Wash. D. C.)*, 189: 726-727, 1975.
- Hsueh, A. J. W., Peck, E. J., Jr., and Clark, J. H. Progesterone antagonism of the estrogen receptor and oestrogen-induced uterine growth. *Nature (Lond.)*, 254: 337-339, 1975.
- Huggins, C., Moon, R. C., and Morii, S. Extinction of experimental mammary cancer. I. Estradiol-17 β and progesterone. *Proc. Natl. Acad. Sci. U. S. A.*, 48: 379-386, 1962.
- Jensen, E. V., De Sombre, E. R., and Jungblut, P. W. Estrogen receptors in hormone-response tissues and tumors. In: R. W. Wissler, T. L. Dao, and S. Wood (eds.), *Endogenous Factors Influencing Host-Tumor Balance*, pp. 15-30. Chicago: University of Chicago Press, 1967.
- Jordan, V. C., Dix, C. J., Naylor, K. E., Prestwich, G., and Roswsby, L. Nonsteroidal antiestrogens: their biological effects and potential mechanisms of action. *J. Toxicol. Environ. Health*, 4: 363-390, 1978.
- Kennedy, B. J. Hormonal therapies in breast cancer. *Semin. Oncol.*, 1: 119-130, 1974.
- Manni, A., Trujillo, J. E., Marshall, J. S., Brodkey, J., and Pearson, O. H. Antihormone treatment of stage IV breast cancer. *Cancer (Phila.)*, 43: 444-450, 1979.
- McGuire, W. L., Carbone, P. P., and Vollmer, E. P. (eds.). *Estrogen Receptors in Human Breast Cancer*. New York: Raven Press, 1975.

16. McGuire, W. L., Raynaud, J. P., and Baulieu, E. E. (eds.). *Progesterone Receptors in Normal and Neoplastic Tissues*. New York: Raven Press, 1977.
17. Mester, J., Martel, D., Psychoyos, A., and Baulieu, E. E. Hormonal control of oestrogen receptor in uterus and receptivity for oovimplantation in the rat. *Nature (Lond.)*, 250: 776-778, 1974.
18. Milgrom, E., Luu Thi, M., Atger, M., and Baulieu, E-E. Mechanisms regulating the concentration and the conformation of progesterone receptor(s) in the uterus. *J. Biol. Chem.*, 248: 6366-6374, 1973.
19. Mouridsen, H., Palshof, T., Patterson, J., and Battersby, L. Tamoxifen in advanced breast cancer. *Cancer Treat. Rev.*, 5: 131-141, 1978.
20. Muggia, F. M., Cassileth, P. A., Ochoa, M., Jr., Flatow, F. A., Gellhorn, A., and Hyman, G. A. Treatment of breast cancer with medroxyprogesterone acetate. *Ann. Intern. Med.*, 68: 328-337, 1968.
21. Robel, P., Levy, C., Wolff, J. P., Nicolas, J. C., and Baulieu, E. E. Réponse à un anti-oestrogène comme critère d'hormonosensibilité du cancer de l'endomètre. *C. R. Hebd. Seances Acad. Sci.*, 287: 1353-1356, 1978.
22. Rubens, R. D., Knight, R. K., and Hayward, J. L. Norethisterone acetate in the treatment of advanced breast cancer. *Eur. J. Cancer (Phila.)*, 12: 563-565, 1976.
23. Segaloff, A., Cunningham, M., Rice, B. F., and Weeth, J. B. Hormonal therapy in cancer of the breast XXIV—effect of corticosterone or medroxy progesterone on the clinical cause and hormonal excretion. *Cancer (Phila.)*, 20: 1673, 1967.
24. Spearman, C. The proof and measurement of two things: part II: correction of "systematic deviations". *Am. J. Psychol.*, 15: 88-101, 1904.
25. Stoll, A. Progestin therapy of breast cancer: comparison of agents. *Br. Med. J.*, 3: 338, 1967.