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Modulation of Natural Killer Cell Activity by Tamoxifen in Stage I Post-Menopausal Breast Cancer*

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Abstract—*Tamoxifen, an antiestrogen which competes for the estrogen receptor, modulates natural killer cell activity* in vivo. *Seventeen post-menopausal stage I breast cancer patients received tamoxifen for* 1 *month and a statistically significant increase in NK activity was demonstrated* (P = 0.0005). *There was a small incremental shift in the number of Leu-11b positive cells. These data demonstrate that tamoxifen functions as a biological response modifier.*

INTRODUCTION

TAMOXIFEN has established itself as an effective form of hormone therapy in hormone receptor positive breast cancer patients in both adjuvant and metastatic settings [1–3]. However, a lack of correlation between hormone receptor positivity and response to tamoxifen has been noted in some trials [1, 4, 5] supporting the concept that tamoxifen may modulate other responses *in vivo*.

Tamoxifen, an antiestrogen which competes directly with estrogens for the cytoplasmic estrogen receptor, has several other properties including prostaglandin synthetase inhibition resulting in reduced production of prostaglandin E [6]. It is also directly cytotoxic to hormone-sensitive human breast cancer cell lines in continuous tissue cultures in the absence of estradiol [7]. Evidence exists that tamoxifen is able to modulate natural killer (NK) activity *in vitro* and *in vivo* [8, 9].

MATERIALS AND METHODS

Eighteen consenting Stage I post-menopausal breast cancer patients, age range 51–72, median age 60 years, who had not received any irradiation or chemotherapy in the preceding 3 months were entered into the study. Tamoxifen, 20 mg (Nolvadex D, ICI Pharma Canada) was given as a single dose orally at night for a period of 4 weeks. Blood was drawn at weeks 0, 4, and 8 for analysis of NK activity.

Peripheral blood mononuclear (PBM) cells were obtained from heparinized blood by density centrifugation over Ficoll-Paque (Pharmacia, Montreal, Quebec). The erythroleukemia line, K-562, was maintained in continuous culture in MEM-alpha and 10% fetal calf serum (Flow Laboratories, Mississauga, Ontario) [10]. An NK assay was performed by incubating 10^4 chromium-51 (Amersham, Montreal, Quebec) labelled target cells (K562) with varying numbers of PBM effector cells for 18 hr at 37° C, in 5% CO_2 air atmosphere in MEM-alpha medium as described previously [11]. The assay was performed at effector-to-target cell ratios of 30 : 1, 10 : 1, 3 : 1, and 1 : 1. One lytic unit was defined as the number of cells required to achieve a particular level of lysis, and the number of lytic units per 10^7 effector cells was determined. Percentage changes in NK activity = lytic units during therapy/lytic units pre-drug × 100%.

Multiple baseline NK levels were not performed as each patient acted as her own control and except for intermittent illness, NK levels have been shown to be relatively consistent [12].

Cell surface markers were assessed using appropriate monoclonal antibodies, to determine the percentage of OKT3 (T-cells), sIg (B-cells) and Leu-11b positive (NK) cells [13].

RESULTS

One patient was inevaluable as, following administration of tamoxifen, she developed a flare reaction with severe lumbar discomfort and was found, on

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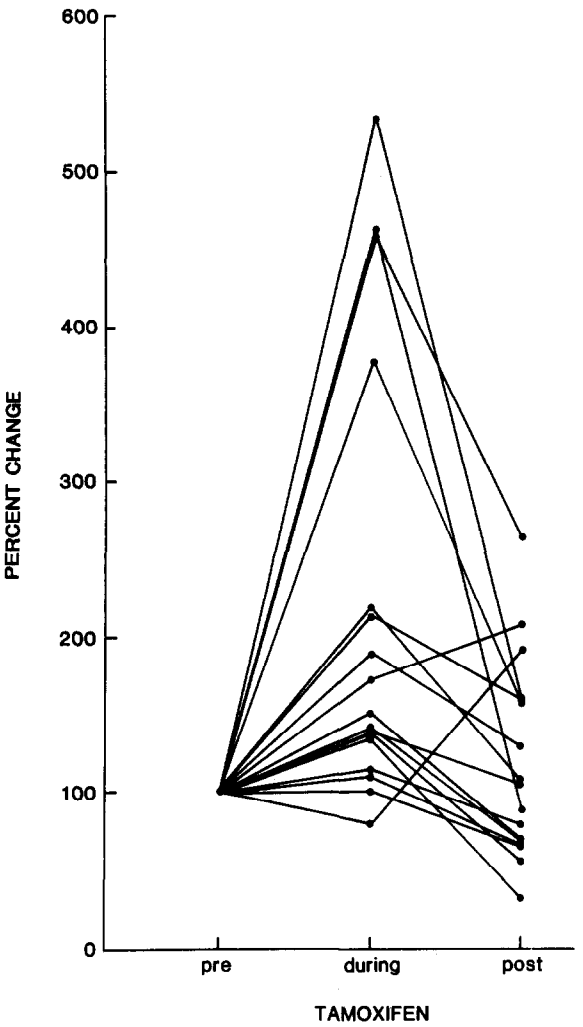

Fig. 1. NK activity as affected by tamoxifen: NK response was assessed prior to, during, and 4 weeks after tamoxifen. The number of lytic units per 10^7 cells was determined for each patient at each data point. The lytic units per 10^7 at the initiation of the study was set at 100% and each subsequent value for each patient was adjusted with respect to the pre-drug level. Hence each patient acted as her own control.

bone scan, to have occult metastatic disease. Indeed her baseline NK was well below normal limits for our laboratory and failed to increase following tamoxifen.

The remaining 17 patients had normal baseline NK activity prior to receiving tamoxifen. Fifteen patients experienced an increase in NK levels over their baseline levels. The average NK activity increased to 221% of pre-drug levels while post-treatment values were 113% of the baseline activities (Fig. 1). The NK value during the treatment phase was significantly elevated compared to the pre-drug level ($P = 0.0005$) and the post-drug levels ($P = 0.0005$) by paired t-test. Hormone receptor status was determined, 8 tumors were estrogen receptor (ER) positive, 3 were ER negative, and 6 were hormone receptor status unknown. No correlation between hormone receptor status and NK response could be made due to the small number of patients studied.

Table 1. Effect of tamoxifen in vivo on NK phenotype

Patient	Pre	During	Post
1	10.2	12.1	14.0
2	12.4	14.2	13.6
3	13.2	18.4	13.2
4	9.2	16.4	18.1
5	10.2	9.8	7.6
6	12.6	14.2	13.9
7	13.6	14.7	12.8
8	12.9	14.3	14.0
9	7.6	8.0	8.1
10	6.0	5.8	10.0
11	13.0	11.2	6.1
12	7.3	7.4	11.2
13	6.0	11.6	8.2
14	6.8	6.8	15.0
15	6.1	8.6	11.4
16	8.8	8.2	8.4
17	11.4	8.6	9.1
Mean ± S.D.	9.8 ± 2.8	11.2 ± 3.6	11.5 ± 3.2

Results expressed as percentage Leu-11b positive cells.

Cell surface markers were analyzed in all 17 evaluable patients. The average percentage of Leu-11b positive cells changed from 9.8% (± 2.8) to 11.5% (± 3.2) after therapy. Although these means and standard deviations illustrate considerable overlaps, by paired t-test statistical analysis, there was a significant increase in the pre to during percentage of positive cells ($P = 0.025$). However, since the values after therapy do not return to baseline, as they did for the NK functional activity (Fig. 1), and the average increase was only 14% (11.2/9.8) compared to 121% for the functional activity and only 10 of 17 patients demonstrated increased Leu-11b positive cells during therapy, we are hesitant to ascribe the significant function increase in NK activity to the change in numbers of Leu-11b positive cells (Table 1). Individually there was no correlation noted between changes in NK activity and the percentage of Leu-11b positive cells. The number of OKT-3 and sIg positive cells were unchanged (data not shown).

DISCUSSION

Our data demonstrate that tamoxifen *in vivo* has the ability to modulate human NK activity. The majority (15 of 17) of the women experienced an increase in NK levels while taking the drug. A small incremental shift in Leu-11b positive cells was demonstrable but this is not felt to be the mechanism by which tamoxifen functions as a biological response modifier.

It has been demonstrated that tamoxifen can enhance murine NK activity *in vivo* [9]. *In vitro* studies on human NK activity have also demonstrated enhancement by tamoxifen [8]. A study assessing the effects of various treatment modalities

on NK activity in breast cancer patients demonstrated tamoxifen alone did not have a deleterious effect on NK activity, whereas progestational therapy was found to suppress NK activity. Therefore not all hormone therapy will enhance NK activity [14].

Estrogen has been shown to be capable of inhibiting human NK cytolysis *in vitro* [15] and the murine systems *in vivo* [16]. However, as tamoxifen does not reduce estrogen levels in post-menopausal patients [17, 18], the enhancement of NK by tamoxifen could not be ascribed to reduction in serum estrogens. Another mechanism of NK modulation observed in pre-menopausal women in the periovulatory phase results in NK depression secondary to elevations of lutenizing hormone (LH) and follicle stimulating hormone (FSH) [19]. *In vitro* modulation of human NK suggests that an inverse relationship exists between LH and NK activity [20]. Tamoxifen *in vivo* does result in reduction of FSH, LH, and prolactin levels in post-menopausal patients [17, 18]. However, the ability to modulate NK *in vitro* by tamoxifen [8] also supports a direct action by the drug. Tamoxifen is known to bind to estrogen receptors. We have previously shown that aminoglutethimide which decreases serum estrogens [21], will enhance NK activity *in vivo* but not *in vitro* [22]; one could speculate that NK cells may have estrogen receptors.

The principal source of prostaglandin production in the immune system is the macrophage. Stimulation by tumour cells may induce excessive production of PGE_2 by macrophages resulting in potent inhibition of lymphoid mitogenesis, cytolysis and antibody production [23]. PGE is thought to serve as a negative feedback in the regulation of macrophages and natural killer cells [24]. Indeed, this documented ability of tamoxifen to reduce PGE production may be the mechanism which resulted in the positive modulation of NK activity in both the *in vivo* and *in vitro* settings.

NK activity would appear to be an important aspect of immune surveillance and have a role in protecting against both malignant and virally infected cells [25]. In breast cancer patients it has been documented that those patients with low NK activity are at a greater risk of developing metastatic recurrence [26]. If tamoxifen is given as adjuvant therapy, the potential enhancement of NK activity may account for the benefit conferred in hormone receptor negative patients [1]. This ability of tamoxifen to act as a biological response modifier by enhancing host NK activity may indicate a wider role for tamoxifen in cancer management.

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