Tamoxifen Chemoprevention of a Hormone-independent Tumor in the Proto-neu Transgenic Mice Model

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Abstract

We evaluated the effect of tamoxifen (TAM) on tumor development in proto-neu transgenic mice that spontaneously develop mammary carcinomas overexpressing the neu protein. These mammary carcinomas are hormone independent because superimposable growth of transplants was observed in females and males.Virgin transgenic mice treated with TAM from 24 weeks of age, i.e., when subclinical mammary tumors are already present, showed a slightly accelerated tumor development. In contrast, transgenic mice treated with TAM starting at 12 weeks of age, when subclinical tumors are not yet present, showed a 50% reduction of tumor incidence. Light microscopy analysis of the mammary gland of these mice revealed an apparently normal ductal branching but a complete regression of the acini. In conclusion, TAM can prevent the occurrence of hormone-independent breast carcinoma if given early enough to inhibit normal cells.

Introduction

Many randomized trials have shown that postsurgical TAM treatment significantly reduces the growth of hormone receptor-positive breast cancer (1), whereas TAM treatment has been found to have no effect on recurrence or mortality rates among women with breast tumors that show low expression or no expression of hormone receptors (2). Recently, the efficacy of TAM for chemoprevention of breast tumors has been evaluated, and the data from the Breast Cancer Prevention Trial (P-1) (3) have demonstrated that TAM administration reduces the incidence of estrogen receptor-positive tumors but not receptor-negative tumors. However, the short observation period and the age distribution of the women enrolled in this study could have masked a possible protection even in the case of hormone receptor-negative tumors. In fact, TAM could act as a chemopreventive agent in two ways: (a) by inhibiting the growth of already present occult tumor cells; and (b) by blocking the cyclical proliferation of the still normal mammary epithelial cells, the target of the neoplastic transformation. In the first case, only hormone receptor-positive tumors are affected, whereas in the second case, all tumors originating from normal breast cells are expected to be prevented. Obviously, in this second case, a longer period of observation is required to detect the chemopreventive effect. Due to the absence of specific information to resolve the issue, to clarify the mechanism of TAM prevention, we evaluated the effects of TAM treatment in proto-neu transgenic mice (4). Although estrogenic properties of TAM have been reported in short tests in mice (5), the adequacy of a murine model to study the effects of TAM treatment has been documented, i.e., the efficacy of long-term treatment to suppress progesterone-induced mammary tumorigenesis (6). The proto-neu transgenic mice spontaneously develop neu-positive tumors (7) that are likely to be hormone independent because repeated cycles of pregnancy and lactation do not accelerate tumorigenesis (4).

Our results show an opposite effect of TAM treatment according to the presence or absence of subclinical tumors at time of treatment start.

Materials and Methods

Mice. A colony of FVB-neuN mice, transgenic line N202 under the MMTV promoter (4, 8), was established in our animal facilities from breeding pairs obtained from Dr. William J. Muller (McMaster University, Hamilton, Ontario, Canada). Mice were maintained under strict inbreeding conditions. The presence of the transgene was routinely checked by PCR on tail DNA using primers hybridizing to vector (5'-ATCGGTGATGTCGGCGATAT-3') and to MMTV sequences (5'-GTAACACAGGCAGATGTAGG-3'). Female mice developed mammary carcinomas with a mean latency time of 40–42 weeks.

Evaluation of Transplanted Tumor Growth. Fourteen-week-old transgenic mice of both sexes received s.c. transplants by injection of an appropriate amount (ranging from 10^3 to 10^6 cells) of mammary tumor cell lines derived from tumors that developed spontaneously in proto-neu transgenic mice. Mice were examined twice a week for tumor growth, and the two perpendicular tumor diameters were recorded for tumor volume evaluation.

Tamoxifen Treatment. In experiment one, 32 proto-neu transgenic mice were randomly divided into two groups, and at 24 weeks of age, the mice were either treated i.p. with 500 µg of TAM diluted in 100 µl of oil for 5 days/week until the mice were 60 weeks old or left untreated. Tumor burden, localization, and volume were recorded weekly for each mouse.

In experiment two, 24 proto-neu transgenic mice were randomly divided into two groups, and at 12 weeks of age, the mice were either treated using the protocol described above or left untreated and evaluated until the mice were 60 weeks old.

Morphological Examination. Mammary tissue samples were obtained from two 16-week-old female virgin transgenic mice treated with TAM for 4 weeks using the protocol described above and from two age-matched control mice. Samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 µm, and stained with H&E for evaluation by light microscopy.

Results

Male and female transgenic mice received s.c. injection of tumor cells obtained from three different mammary tumor cell lines that developed spontaneously in proto-neu transgenic mice. All tumors grew in the mice independent of sex, and no differences between male and female mice were observed in the latent period, which ranged from 12–25 days, depending on the cell line and the tumor growth rate (data not shown). This observation indicates the hormone independence of these tumors.

The effect of TAM was first evaluated in transgenic mice treated at...
24 weeks of age, i.e., when spontaneous mammary tumors start to be subclinically detectable (9). Sixteen transgenic female virgin mice were treated with TAM, and the tumor incidence was compared with that which occurred in 16 untreated age-matched mice. A modest but significant acceleration of tumor development in TAM-treated mice was observed (Fig. 1A). Indeed, 50% of the TAM-treated mice developed tumors by week 38, whereas latency in the control group was 42 weeks ($P < 0.02$). No differences in the number of tumors/mouse or the tumor growth rate were observed (data not shown).

The chemopreventive effects of TAM were then evaluated in younger mice when subclinical tumors were not yet present (7). Twenty-four transgenic female virgin mice (12 weeks old) were divided into two groups and either treated with TAM using the protocol described above or left untreated. Tumor development was significantly inhibited in treated mice as compared to the control group; at 55 weeks of age, all untreated mice had developed mammary tumors, whereas about 50% of TAM-treated mice remained tumor free ($P = 0.0003$; Fig. 1B). Light microscopy analysis of the mammary gland of 16-week-old sexually mature female virgin transgenic mice treated with TAM for 4 weeks revealed apparently normal ductal branching but a complete regression of the acini compared to mammary glands from the untreated control mice, which clearly showed the presence of both ducts and terminal lobules (Fig. 2).

**Discussion**

Mammary carcinomas, which develop spontaneously in transgenic mice due to the presence of the proto-neu transgene, are not regulated by estrogens because superimposable growth of s.c. transplants was observed in females and males. No inhibitory effect of TAM on tumorigenesis was observed in mice treated beginning at 24 weeks of age, when subclinical tumors are already present; instead, a slightly accelerated tumor development was observed. On the contrary, a

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**Fig. 1.** A, effect of TAM treatment in 24-week-old transgenic mice as compared to untreated transgenic mice. $\boldsymbol{\square}$, 16 treated mice; $\boldsymbol{\blacklozenge}$, 16 untreated mice. B, effect of TAM treatment in 12-week-old transgenic mice as compared to untreated transgenic mice. $\boldsymbol{\square}$, 12 treated mice; $\boldsymbol{\blacksquare}$, 12 untreated mice.

**Fig. 2.** Light microscopy images of mammary gland sections obtained from 16-week-old transgenic mice (samples were stained with H&E). Sections are from a mouse treated with TAM for 4 weeks (C and D), showing a marked loss of acini, and from a control transgenic mouse (A and B), showing normal development of acini (arrows). Magnification: A and C, $\times$100 (bar, 100 μm); B and D $\times$250 (bar, 40 μm).
strong protective effect of TAM with respect to tumor incidence was observed in mice treated beginning at 12 weeks of age, when transformed cells are not yet present.

The absence of TAM protection observed in mice treated at 24 weeks of age, at the early tumor formation, argues against the possibility that tumors arise in mice as hormone dependent and that the hormone independence observed in transplants is acquired during tumor progression. Therefore, in our model, TAM, when given early enough, also displays a protective effect on tumorigenesis of hormone-independent carcinomas. Our results indicate that the mechanism of TAM chemoprevention involves a reduction in the number of potential target cells for transformation, in keeping with the clear loss of terminal acini cells in the mammary glands of mice treated with TAM beginning at 12 weeks of age. The high sensitivity of acini to TAM is consistent with their high responsiveness to estrogens. Another possibility, namely, that the chemopreventive effect of TAM is due to a down-regulation of the MMTV promoter, can be excluded because this promoter has been shown not to be down-modulated by TAM (10). In keeping with this, tumor cells derived from treated and untreated mice showed the same levels of neu protein on the cell membrane, as determined by flow cytometric analysis. Also, a tumor cell line derived from a transgenic mouse showed no increase in transgene expression after in vitro treatment with TAM (data not shown).

The modest acceleration of tumor development in mice treated with TAM beginning at 24 weeks of age might reflect the ability of this drug to stimulate neu-positive cells directly, by binding neu membrane receptor (11), or indirectly, by inducing the production of factors such as tumor growth factor β (12, 13). In keeping with our findings, a detrimental effect of TAM adjuvant treatment in women with HER2-positive breast carcinoma has been reported (14).

In conclusion, TAM can exert its chemopreventive effect by hampering the growth of occult hormone-dependent tumors and also by interfering with the initiation of tumors through a decrease in the number of normal cells at risk of transformation. Although additional studies are needed, our results suggest that in young women with an increased risk for breast cancer, the timing of TAM adjuvant treatment is of paramount importance.

References