Advances in Brief

Induction of Tamoxifen-dependent Rat Mammary Tumors

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Abstract

We have observed previously that animals given tamoxifen (TAM) and the carcinogen dimethylbenzanthracene develop exclusively hormone-independent tumors. Since our data implied that the TAM-associated tumors were different from control tumors, we undertook studies to examine the role of TAM in the induction and growth of these tumors. Following cessation of TAM administration, almost one-third [29.0 ± 1.7% (SEM)] of the tumors regressed and more tumors appeared. Resumption of TAM administration resulted in regrowth of some tumors and regression of the new tumors. These studies demonstrate that some of the TAM-associated tumors are actually dependent upon TAM for growth, while the appearance of new tumors suggests that TAM does not totally prevent tumor formation but may only delay it.

Introduction

While TAM has repeatedly been shown to be an effective anticancer agent in both animal and clinical studies (1-4), several studies have reported that human tumors may develop resistance to this agent (5-7). Previous work from this laboratory has also demonstrated that tumors induced in the presence of TAM are exclusively hormone independent and possess characteristics distinct from those of control hormone-dependent and independent tumors (8). These TAM-associated tumors possessed significantly lower levels of the estrogen receptor and grew at a rate that was more than 3 times faster than that of hormone-independent tumors in control animals (8). For these reasons, we undertook studies to examine the role of tamoxifen in the induction and growth of these hormone-independent tumors.

Materials and Methods

Chemicals. The carcinogen DMBA and tamoxifen citrate were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals. Adult female Sprague-Dawley rats (55 days old) were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Animals were maintained in a temperature-controlled room on a 12 h:12 h day:night cycle with free access to food and water. The reproductive cycles of the animals were monitored by daily vaginal smears. Administration of carcinogen and all surgery were performed under light ether anesthesia. Animals were sacrificed by CO₂ asphyxiation.

Tumor Induction. The carcinogen DMBA was dissolved in sesame oil and administered by gavage under light ether anesthesia once a week for 4 weeks (5 mg/rat/week; total dose, 20 mg) as modified from Isaacs (9). This protocol results in a greater number of tumors with characteristics identical to those of the original Huggins model (10, 11). TAM (10 mg/kg body weight in sesame oil) was injected s.c. coincident with DMBA administration. Tumor number and volume were determined weekly starting at week 3 following the last coadministration of carcinogen and TAM. Tumor volume was calculated according to:

\[ V = \frac{4}{3}\pi R_1^2 R_2 \]

In some experiments selected animals received additional periodic administrations of TAM (10 mg/kg body weight) and monitoring of tumor growth and number was continued. Each experiment had 10 animals/group. In total these experiments were conducted with 60 rats possessing 183 tumors (46 tumors were found in animals receiving tamoxifen). Experiments were performed at least twice and all data are reported as mean ± SEM.

Results

As in our earlier experiments the coadministration of TAM and DMBA resulted in a dramatic reduction in the number of tumors observed, but ultimately 61.8 ± 8.3% of TAM-treated rats developed tumors (range, 45-70%). However, following the cessation of TAM administration, several tumors were observed to regress (Fig. 1). In all 29 ± 1.7% of TAM-associated tumors regressed significantly; in contrast, only 4.25 ± 0.05% of control tumors regressed spontaneously by more than 20% over the course of these experiments (P < 0.005). Tumor regression initiated prior to any resumption of regular reproductive cycles, but occasional proestrus and estrus smears were observed. Subsequent sacrifice of these animals revealed ovarian wet weights significantly lower than those of control animals (60.3 ± 6.4 mg in TAM-treated animals versus 100.0 ± 4.3 mg in controls; P < 0.001). Estrogen receptor analysis of TAM-associated tumors revealed a 56.7 ± 2.6% reduction in estrogen receptor concentration versus control tumors, similar to that observed in earlier experiments. Readministration of TAM to selected animals resulted in significant regrowth of these tumors (Fig. 2). The TAM-associated tumors that had regressed totally in the absence of TAM did not reappear upon readministration of TAM. The remaining tumors in the TAM group maintained growth regardless of hormonal status and were classified as hormone independent.

In addition to the hormone-independent tumors which we observed in association with TAM, new tumors began to appear within 5 weeks of cessation of TAM treatment. Of these new tumors, 42.8% were subsequently classified as hormone dependent, because following readministration of TAM or ovariectomy these new tumors regressed. While these new tumors appeared prior to the resumption of regular estrous cycles, reproductive cycles resumed shortly and upon sacrifice ovaries were observed to have active follicles in contrast to TAM-treated ovaries which are essentially regressed.

Discussion

As in our earlier studies we observed solely hormone-independent tumors in TAM-treated rats with comparable numbers of animals developing tumors. Our current studies further demonstrate that some of the hormone-independent tumors that develop in the presence of tamoxifen are actually dependent upon tamoxifen for the maintenance of growth, in that they regress following cessation of TAM treatment and begin to regrow upon readministration of TAM. Furthermore, while TAM-treated animals have significantly fewer tumors than controls, as TAM is cleared from the animals, new hormone-dependent tumors begin to appear suggesting that TAM is serving to inhibit growth in these tumors rather than prevent tumor formation.
Because of the altered growth rate of the hormone-independent tumors in our previous experiments, we believed that the tamoxifen-associated tumors were different from control hormone-independent tumors. Moreover, our earlier studies found that these tamoxifen-associated tumors had lower estrogen receptor levels than either hormone-dependent or independent tumors in control animals. In the current studies we observed that almost one-third of all the tumors that appear in tamoxifen-treated animals are actually dependent upon that agent for growth. Actual regression of tumors usually appeared within 6 weeks after cessation of treatment; this observation may be due in part to the prolonged half-life of this agent in rats (12).

While tamoxifen was shown to dramatically reduce the appearance of tumors in these rats, this effect was diminished following discontinuation of treatment. Jordan et al. (13) have previously reported that tamoxifen acts, in part, to delay appearance of rat mammary tumors. Similarly, in the mouse, tamoxifen was not totally effective in eliminating breast cancer (14). Therefore, while tamoxifen is effective at reducing the incidence of mammary tumors in animal models, it is not completely effective and acts to delay the appearance of some tumors. Continued administration of tamoxifen is, therefore, necessary to maintain the antitumorigenic effects. In human breast cancer cell lines, the continued presence of TAM eventually results in TAM resistance in a subpopulation of the cells. Similarly, in some women treated with this drug a certain degree of resistance to TAM may develop. Osborne et al. (6) have demonstrated that the resistance may actually represent an altered metabolism of TAM resulting in resumption of tumor growth that is stimulated by TAM. While some investigations have correlated the degree of antiestrogen resistance with estrogen receptor concentrations (15); others have reported that TAM resistance may become manifest through the antiestrogen binding site (16). It is well recognized that both this animal model and human breast cancers possess the antiestrogen binding site (17, 18), therefore, we are uncertain whether the tamoxifen dependence is mediated through the estrogen receptor, through the antiestrogen binding site, or through altered metabolism of tamoxifen. Recent evidence in human breast cancer both in vitro and in vivo suggests that TAM and its metabolites can act through estrogen receptor-dependent and -independent mechanisms (19, 20).

The effects that we observe in the rat occur over a matter of weeks; since breast tumor formation in humans may take 8–10 years before the tumor reaches the level of detection (4), effects similar to what we have observed may take years to develop in women. Both the development of TAM-dependent tumors and the incomplete preventive action for this drug should be considered in the ongoing prophylactic clinical trials with this agent. It is unknown whether TAM-dependent tumors will occur in women treated with this drug, nor is it known whether continued administration may increase the chance of developing TAM-dependent tumors. As mentioned earlier, resistance to TAM has been reported in women under prolonged treatment. From the above studies, it is logical to assume that some of these resistant tumors may actually be TAM dependent as observed in our studies.

While it appears evident that TAM does play a role in maintaining the growth of these hormone-independent tumors in this model, it remains unknown whether TAM is playing a role in the induction of these specific hormone-independent tumors. However, there is no question from our studies that tumors that appear during TAM administration are more aggressive than control tumors and some rely upon this agent for growth. Furthermore, the effects of this compound in animal models have repeatedly been found to be analogous in humans. Therefore, human cancers that appear during TAM therapy
should be characterized to determine whether this drug is playing a role in the induction and maintenance of human tumors.

References


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