Eur J Cancer Clin Onol, Vol. 17, No. 12, pp. 1255–1258, 1981
Printed in Great Britain.

0277-5379/81/121255-04$02.00/0

Effect of an Estrogen Antagonist (Tamoxifen) on the Initiation and Progression of γ-Irradiation-Induced Mammary Tumors in Female Sprague–Dawley Rats*

CLIFFORD W. WELSCH,†‡ MARGARET GOODRICH-SMITH,† CAROLYN K. BROWN,† NANCY MIGLORIE and KELLY H. CLIFTON§

†*Department of Anatomy, Michigan State University, East Lansing, Michigan* 48824 *and* §*Department of Human Oncology, University of Wisconsin, Madison, Wisconsin* 53792, *U.S.A.*

Abstract—*One hundred and sixty-eight female Sprague–Dawley rats were divided among* 4 *groups* (42 *rats/group*) *and were irradiated with* 400 rad [^{137}Cs] γ *rays to the whole body when* 59 days *old. The estrogen antagonist tamoxifen was injected s.c. daily from* 29–89 days *of age* (*Series* 1) *or from* 89–149 days *of age* (*Series* 2), *prior to the onset of palpable mammary tumors. Two groups of rats were injected with the diluent and served as controls. All rats were palpated bi-weekly for mammary tumors and killed* 37 weeks *after γ-irradiation. The number of mammary carcinomas which developed in each group and their significance levels were: series* 1, *controls,* 31; *tamoxifen treated,* 11, $P < 0.01$; *series* 2, *controls,* 33; *tamoxifen treated,* 17, $P < 0.01$. *Tamoxifen treatment did not significantly influence the incidence of benign mammary tumors (fibroadenomas) nor the rate of body weight gain. Thus, the effective use of an estrogen antagonist for the prophylaxis of radiogenic mammary carcinomas has been demonstrated in this study.*

INTRODUCTION

ONE OF the most important advances during the past decade has been the development of anti-hormonal drugs for the treatment of a number of endocrine-related pathologies, not the least of which is human breast neoplasia. Tamoxifen (ICI 46,747) [trans-1-(*p*-β-dimethylaminoethoxyphenyl)1,2 diphenyl but-1-ene], a non-steroid belonging to the triphenylethylene class of compounds, is a potent estrogen antagonist in rats and humans[1, 2]. It is one of the most widely used anti-estrogens for the treatment of human breast cancer because of its effective oncolytic activity and its relatively insignificant side effects[3]. Tamoxifen antagonizes the action of estrogen by competing for cytoplasmic receptor complex, by altering the association of the receptor complex and nuclear binding sites and/or by interfering with the regeneration of the cytoplasmic receptor[4].

In 1971, Terenius [5] and Heuson and colleagues [6] demonstrated that treatment of female rats with an estrogen antagonist (MER-25, U-11, 100A) would significantly suppress 7,12-dimethylbenzanthracene (DMBA)-induced mammary carcinogenesis. These observations were confirmed and extended by Jordan[7], who reported the efficacy of tamoxifen in this neoplastic process. The endocrine dependence of the polycyclic hydrocarbon-induced rat mammary carcinoma is now well known[8].

Mammary tumors can be induced in rats by exposure to ionizing irradiation. Reports by Clifton and Stridharan[9], Shellabarger[10] and Yokoro *et al.*[11] have provided evidence that these neoplasms are reponsive to endocrine

Accepted 6 May 1981.
*This study was supported by NIN contract NO1-CB-74090.

‡Reprint requests to: Dr. Clifford W. Welsch, Department of Anatomy, Michigan State University, East Lansing, MI 48824, U.S.A.

secretion. The effect of anti-estrogens on the genesis of such tumors is described in this report.

MATERIALS AND METHODS

One hundred and sixty-eight female Sprague–Dawley rats (Spartan Research Animals, Inc., Haslett, MI) were housed in a temperature-controlled (24 ± 1°C) and light-controlled (14 hr/day) room and fed Wayne Lab Blox (Allied Mills, Inc., Chicago, IL) and water *ad libitum*. At 59 days of age, all rats were irradiated with 400 rad [^{137}Cs] γ-rays to the whole body in a four pi irradiator at a dose rate of 1000 rad per min. The estrogen antagonist tamoxifen was injected s.c. daily to 2 groups of rats (42 rats/group) from 29–89 days of age (series 1) or from 89–149 days of age (series 2) prior to the onset of palable mammary tumors. In series 1 and 2, the rats were injected with 200 μg tamoxifen dissolved in 0.1 ml peanut oil for the first 35 days and 50 μg tamoxifen in 0.1 ml for the last 25 days. Two groups of rats (42 rats/group) were injected s.c. with peanut oil only and served as controls.

All rats were palpated bi-weekly for mammary tumors. The tumors were removed, fixed, sectioned, stained with hematoxylin and eosin, and examined histologically. All rats were killed 37 weeks after irradiation. Ovaries, uteri, adrenals and pituitaries were removed and weighed. In rats of series 2, 24 hr after the last injection of tamoxifen, a 1.0 ml of blood was obtained by cardiac stab from each of 10 tamoxifen-treated rats and from 10 control rats and analyzed by radioimmunoassay for prolactin (National Institute of Arthritis, Metabolism and Digestive Diseases rat prolactin radioimmunoassay kit). Mean differences between blood prolactin levels and organ weights were evaluated statistically by Student's t test. Mammary tumor incidence was analyzed by χ^2 analysis.

RESULTS

Treatment of female rats with tamoxifen for 60 days, i.e., 30 days before and 30 days after irradiation (series 1) or for 60 days beginning 30 days after irradiation (series 2), significantly ($P < 0.01$) reduced the incidence of mammary carcinomas (Figs 1 and 2, Table 1). No significant effect of anti-estrogen on the incidence of benign mammary tumors (fibroadenomas) was observed (Table 1). No significant differences in mean uterine, ovarian, adrenal and pituitary gland weights were observed between control and tamoxifen-treated rats killed 33 (series 1) and 25 (series 2) weeks after cessation of tamoxifen treatment. Serum levels of prolactin (ng/ml) were not significantly different in tamoxifen-treated rats (165.1 ± 33.5) when compared with control rats

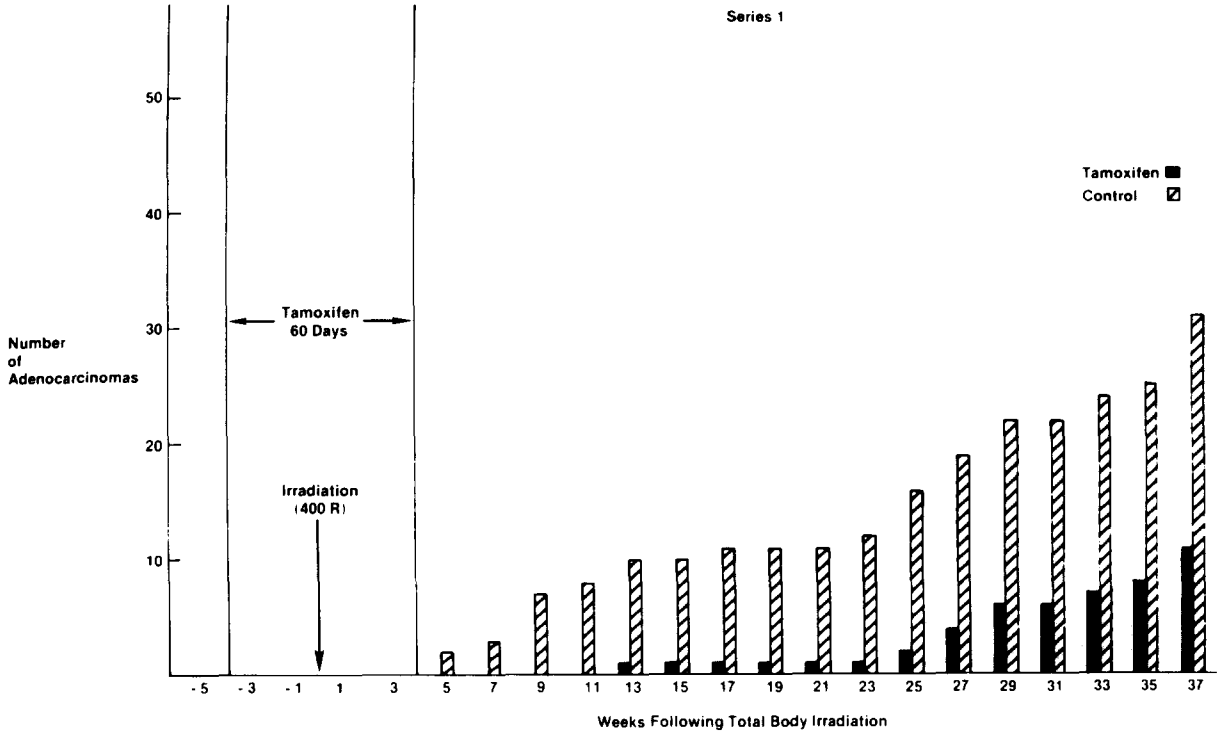

Fig. 1. Effect of tamoxifen treatment (29–89 days of age) on the genesis of mammary adenocarcinomas in irradiated female Sprague–Dawley rats. (42 rats/group) Control vs. tamoxifen, $P < 0.01$.

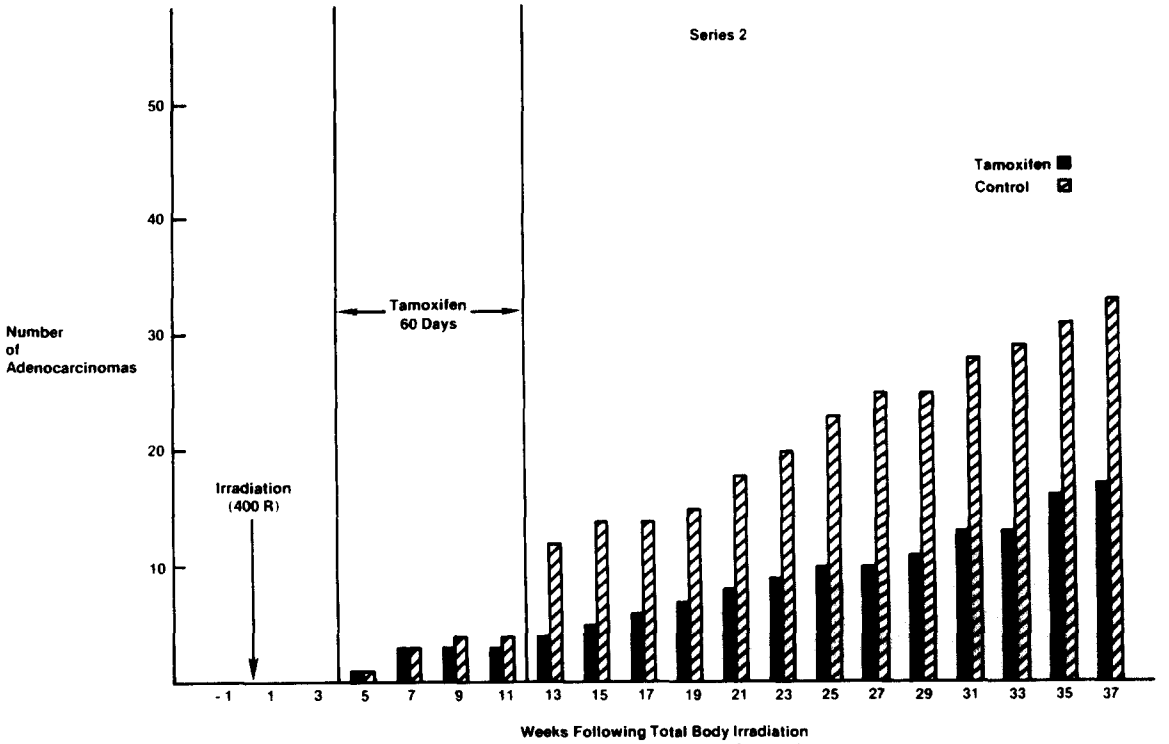

Fig. 2. Effect of tamoxifen treatment (89–149 days *of age*) *on the genesis of mammary adenocarcinomas in irradiated female Sprague–Dawley rats.* (42 *rats/group*) *Control vs. tamoxifen,* $P < 0.01$.

Table 1. Effect of tamoxifen on the genesis of mammary tumors in irradiated female Sprague–Dawley rats

	Number of rats at onset of study	Number of mammary tumors at termination of study* adenocarcinomas	fibroadenomas	Percent of rats with mammary tumors adenocarcinomas	fibroadenomas
Series 1†					
Control	42	31[a]	20[c]	23/42 (55%)	14/42 (33%)
Tamoxifen	42	11[b]	24[d]	10/42 (24%)	14/42 (33%)
Series 2‡					
Control	42	33[a]	16[c]	22/42 (52%)	11/42 (26%)
Tamoxifen	42	17[b]	11[d]	15/42 (36%)	10/42 (24%)

*All rats were sacrificed 37 weeks after irradiation.

†400 R of total body irradiation at 59 days of age, Tamoxifen was administered daily at 29–89 days of age.

‡400 R of total body irradiation at 59 days of age, Tamoxifen was administered daily at 89–149 days of age.

[a/b] $P < 0.01$.

[c/d] P = no significance. Mean latency periods of fibroadenoma appearance were: series 1, controls, 261 days, tamoxifen-treated, 256 days; series 2, controls, 252 days, tamoxifen-treated, 257 days. Thus tamoxifen treatment also did not significantly influence mean latency period of benign mammary tumor appearance.

(174.4 ± 38.0). Tamoxifen, at the dose levels used, did not significantly alter body weight gains. Non-tumor related mortality was minimal and comparable among the 4 groups of rats.

DISCUSSION

Mammary tumors induced in rats by ionizing irradiation have received less attention than those induced by polycyclic hydrocarbons. Morphologically, the mammary tumors which follow γ-irradiation in rats are primarily adenocarcinomas resembling the mammary neoplasms induced by DMBA, although numerous benign mammary tumors, usually with long latency periods, do appear in irradiated rats[10, 12]. Mammary adenocarcinomas in rats induced by irradiation have also been reported to be prolactin [11] and ovarian hormone [13] responsive.

The results of this study clearly show that the genesis of radiation-induced mammary carcinomas in rats can be substantially suppressed by the administration of tamoxifen for periods prior to the overt emergence of these neoplasms. The frequency of occurrence of benign mammary neoplasms, on the other hand, were not affected by treatment with the estrogen antagonist. This suggests that the endocrine conditions which predispose to the genesis of mammary carcinomas after irradiation are distinctly different from those which predispose to benign mammary neoplasms. The lack of a significant effect of tamoxifen on serum prolactin levels provides evidence that the activity of the anti-estrogen is direct, i.e., not mediated via an altered secretion of prolactin.

Acknowledgements—We thank Dr. David H. McCurdy, ICI Americas Inc. for a generous supply of tamoxifen (ICI 46,474).

REFERENCES

1. JORDAN VC. Antiestrogenic and antitumor properties of tamoxifen in laboratory animals. *Cancer Treat Rep* 1976; **60**: 1409–1420.
2. LUNAN CB, KLOPPER A. Antioestrogens: a review. *Clin Endocrional (Tokyo)* 1975: **4**: 551–572.
3. LEGHA SS, CARTER SK. Antiestrogens in the treatment of breast cancer. *Cancer Treat Rev* 1976; **3**: 205–216.
4. KATZENELLENBOGEN BS, BHAKOO HS, FERGUSON ER, LAN NC, TATEE T, TSAI TL, KATZENELLENBOGEN JA. Estrogen and antiestrogen action in reproductive tissues and tumors. *Recent Prog Horm Res* 1979; **35**: 259–300.
5. TERENIUS L. Anti-oestrogens and breast cancer. *Eur J Cancer* 1971; **7**: 57–64.
6. HEUSON JC, WAELBROEK C, LEGROS C, GALLEZ G, ROBYN C, L'HERMITE N. Inhibition of DMBA-induced mammary carcinogenesis in the rat by 2-Br-α ergocryptine (CB 154), an inhibitor of prolactin secretion, and by nafoxidine (U11, 100A), an estrogen antagonist. *Gynecol Invest* 1971; **2**: 130–137.
7. JORDAN VC. Effect of tamoxifen (ICI 46,474) on initiation and growth of DMBA-induced rat mammary carcinomata. *Eur J Cancer* 1976; **12**: 419–424.
8. WELSCH CW, NAGASAWA H. Prolactin and murine mammary tumorigenesis: A review. *Cancer Res* 1977; **37**: 951–963.
9. CLIFTON KH, SRIDHARAN BN. Endocrine factors and tumor growth. In: BECKER FF, ed. *Cancer*. New York. Plenum Press, 1975: Vol. 3, pp. 249–285.
10. SHELLABARGER GJ. Modifying factors in rat mammary gland carcinogenesis. In: YUHAS JM, TENNANT RW, REGAN JD, eds. *Biology of Radiation Carcinogenesis*. New York: Raven Press, 1976: pp. 31–43.
11. YOKORO K, NAKANO M, ITO A, NAGAO K, KODAMA Y, HAMADA K. Role of prolactin in rat mammary carcinogenesis: Detection of carcinogenicity of low-dose carcinogens and of persisting dormant cancer cells. *J Natl Cancer Inst* 1977; **58**: 1777–1783.
12. CLIFTON KH, DOUPLE EB, SRIDHARAN BN. Effects of grafts of single anterior pituitary glands on the incidence and type of mammary neoplasm in neutron- or γ-irradiated Fischer female rats. *Cancer Res* 1976; **36**: 3732–3735.
13. TAKIZAWA S, NAITO Y, WATANABE H, HIROSE F. Effect of ovariectomy on X-ray carcinogensis in rats. *Gann* 1976; **69**: 353–360.