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THE ANTITUMOR POTENCY OF PROGESTERONE ANTAGONISTS IS DUE TO THEIR DIFFERENTIATION POTENTIAL

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Summary—A new therapy for the progesterone receptor positive mammary carcinoma may be the treatment with progesterone antagonists. This new class of antihormones causes a strong inhibition of tumor growth comparable to the potency of ovariectomy in a panel of experimental mammary carcinomas. The mechanisms of the strong tumor-inhibiting action of progesterone antagonsits on experimental mammary carcinomas mainly depends on a progesterone receptor mediated process leading to induction of terminal differentiation and a blockade of the cell cycle. To further characterize the antitumor mechanism of progesterone antagonists we analyzed the effects of Onapristone and ZK 112.993 on DMBA- and MNU-mammary tumors of the rat and MXT-tumors of the mouse after different therapy intervals. These hormone-dependent mammary tumors normally display intraductal growth in papillary, cribiform or solid formations, whereas after treatment periods of 2–6 weeks with progesterone antagonists they displayed dysplastic ductal and acinous formations, usually filled with secretory material. Whereas tumor size, mitotic index, and the grade of tumor malignancy decreased distinctly, the volume fraction of glandular structures in the tumors as well as the appearance of apoptosis increased 3-fold compared to the controls. In addition, the mammary glands of progesterone antagonist treated animals showed the morphological features of differentiation with the appearance of secretory activity. Interestingly, the staining pattern of some of the lectins used, especially UEA 1 binding pattern, fits to the concept of differentiation since recent studies revealed a higher degree of fucosylation only in benign lesions of human breast cancers. Therefore, these data underline the concept of a differentiation potential of progesterone antagonists on progesterone receptor positive mammary carcinomas.

INTRODUCTION

A new therapy for the progesterone receptor positive mammary carcinomas may be the treatment with progesterone antagonists like Onapristone (Fig. 1). This new class of antihormones causes a strong inhibition of tumor growth, comparable to the potency of ovariectomy in a panel of experimental mammary carcinoma models [1–5]. Their mechanism depends on a progesterone receptor mediated effect [3], leading to the induction of terminal differentiation [3, 4] with a blockade of the cell cycle [6]. To further examine the mechanism and the characteristics of terminal differentiation, the progesterone antagonist treated mammary carcinomas and mammary glands were analyzed by use of morphometrical, lectin histochemical and ultrastructural methods.

Two hormone-dependent chemically-induced mammary tumor models of the rat (DMBA and MNU) and a transplantable hormone-dependent mammary tumor model of the mouse (MXT) were chosen for this study [4, 5]. After treatment periods of 2–6 weeks with antiprogestins the tumors and mammary glands of the animals were compared to untreated controls, ovariectomy and animals treated with the clinically established endocrine therapies. As antiprogestins Onapristone (ZK = 98.299) and ZK 112.993 were used. To describe the histological degree of tumor differentiation, grading was evaluated, according to the proposals of Bloom and Richardson [7], modified by Elston [8]. The volume density of the different components of tissue was analyzed by the point-counting method [9]. A minimum of 12 tumors in every group was judged by two

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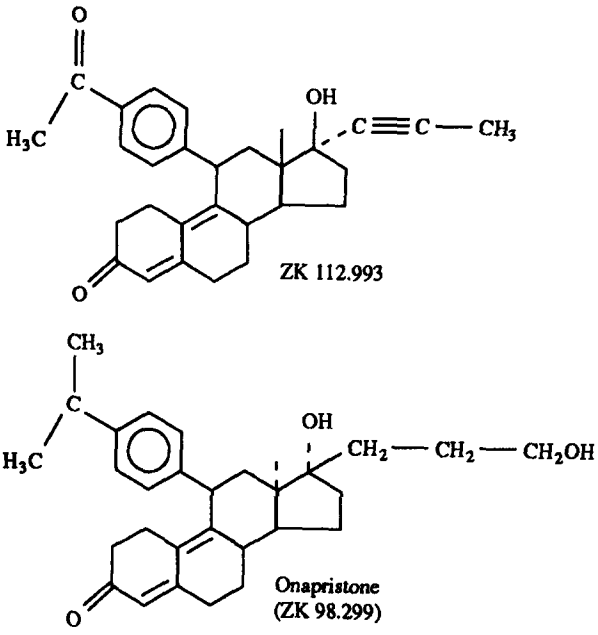


Fig. 1. Chemical structure of the progesterone antagonists Onapristone (=ZK 98.299) and ZK 112.993.

experts who did not know the experimental group. More than 10 sections were analyzed from every tumor. The α-fucose specific Ulex europaeus (UEA-I) lectin binding was done on paraffin embedded material by the indirect PAP-method. Tumor areas are expressed as means in mm^2. Differences in the means of morphometric data were analyzed for significance by the Dunnet-test. The significance limit was set at $P < 0.05$.

RESULTS

Whereas only the mammary carcinomas treated with progesterone antagonists displayed ductal and acinous structures filled with secretory material, the carcinomas of the other groups grow intraductal in papillary, cribriform or in solid formations of epitheliosis (Fig. 2, see p. 205). These tumors only occasionally showed signs of infiltration. The loss of epitheliosis (Figs 4 and 5) is balanced by an increased volume density and glandular lumen (Figs 2, 3a and b, 4a and 5b). In contrast, after ovariectomy and treatment with tamoxifen a higher amount of connective tissue was detected. The decrease of tumor area, weight (Figs 3, 4 and 5) and grade of malignancy characterizes the treatment with progesterone antagonists. No differentiation effects of DES in the tumors were observed (Figs 5a, b and c). In addition, after antiprogestin treatment the mammary tumors show less UEA-I binding (Fig. 4d, see p. 206). A typical sign of hormone therapy is the increased amount of apoptosis (Figs 3c and d). Interestingly, the mammary glands of the same animals reflect differentiation towards acinous structures showing secretory activity (Figs 5d, see p. 206, e and f). Such reaction patterns were observed neither in ovariectomized nor in tamoxifen treated animals.

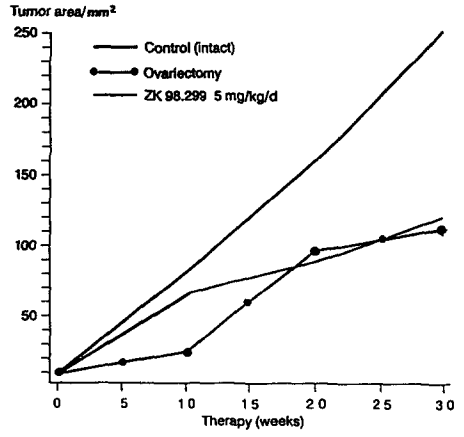


Fig. 3. Growth pattern of the hormone-dependent MXT(+) mammary carcinoma of the mouse.

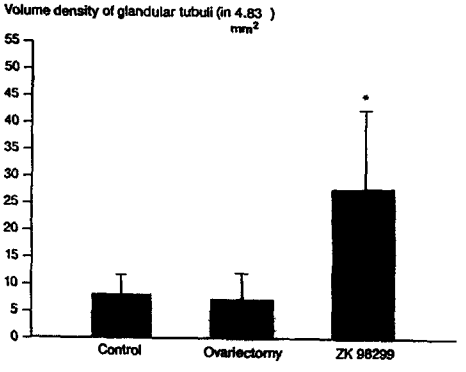


Fig. 3a. Volume density of glandular structures in the MXT(+) mammary carcinoma.

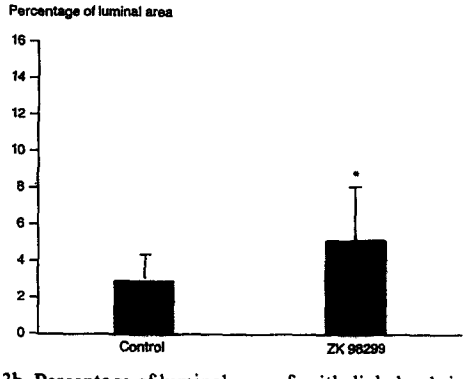


Fig. 3b. Percentage of luminal area of epithelial glands in the MXT(+) mammary carcinoma

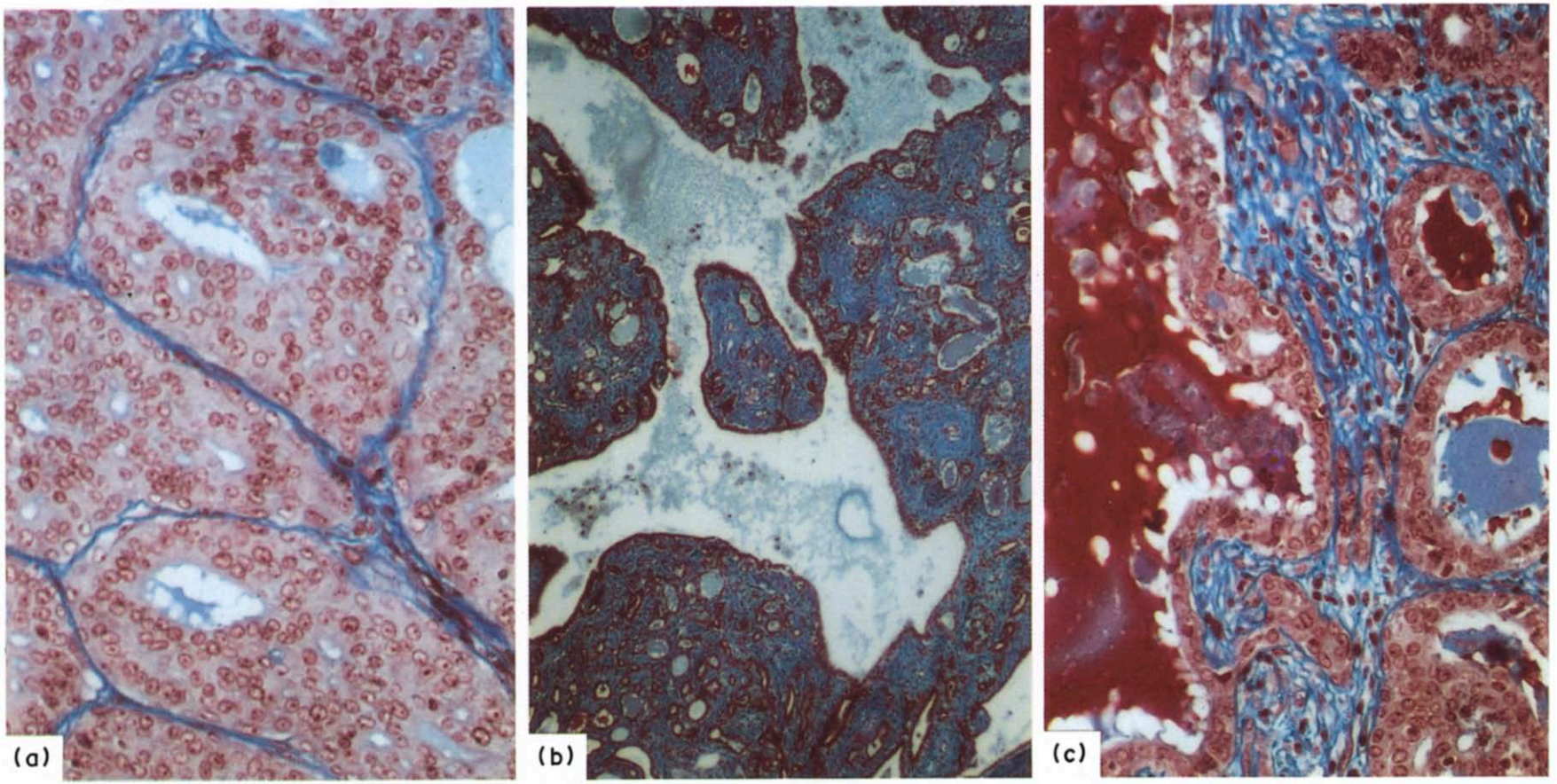


Fig. 2. (a) Untreated DMBA-induced mammary carcinoma of the rat (Azane/ × 280). (b) DMBA-induced mammary carcinoma after ovariectomy. Note: amount of connective tissue (Azane/ × 70). (c) Onapristone treated DMBA-induced mammary carcinoma with secretory activity in the epithelium (Azane/ × 280).

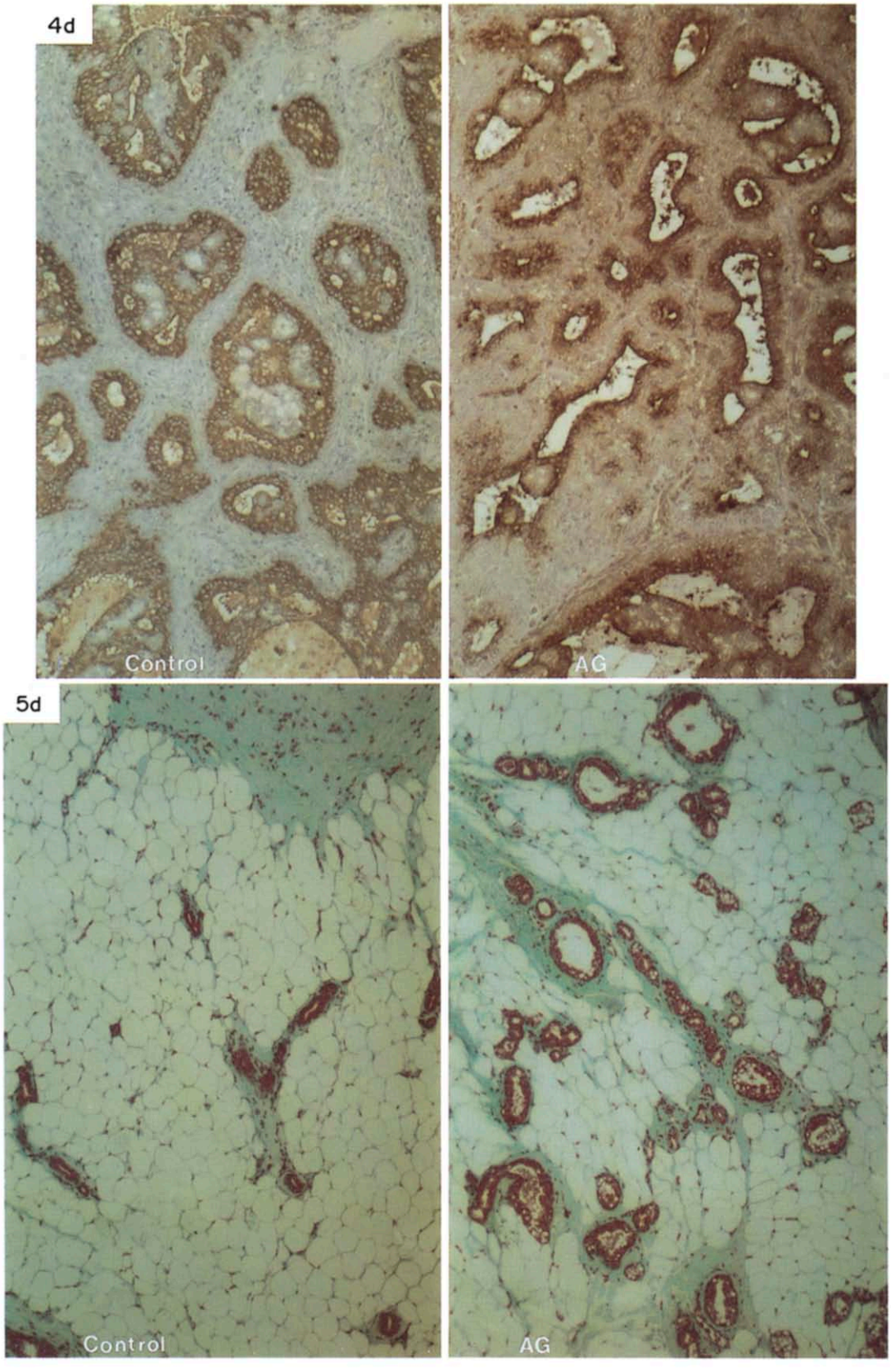


Fig. 4d. α-Fucose specific UEA-I binding pattern in an untreated (control) and with antiprogestin (=AG) NMU-induced mammary carcinoma. The treated tumors display positive cells only within the luminal layer of the epithelium (diaminobenzidin ×110).

Fig. 5d. Mammary glands of DMBA tumor-bearing animals. Untreated (control) and with progesterone antagonist (=AG) treated mammary gland. Note: degree of differentiation and intraepithelial secretory vacuoles (Masson-Goldner ×70)

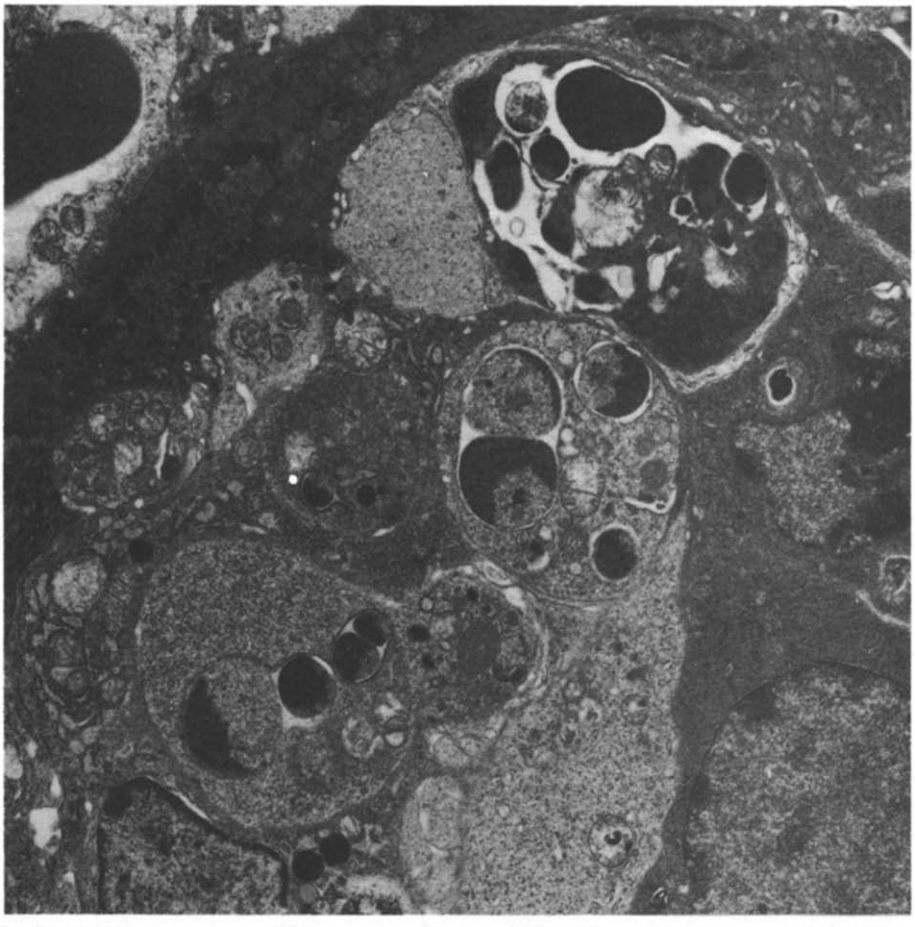


Fig. 3c. Characteristic apoptotic cell bodies of the tumor epithelial cells in Onapristone treated MXT(+) mammary tumor: transmission electron microscopy ×9 360.

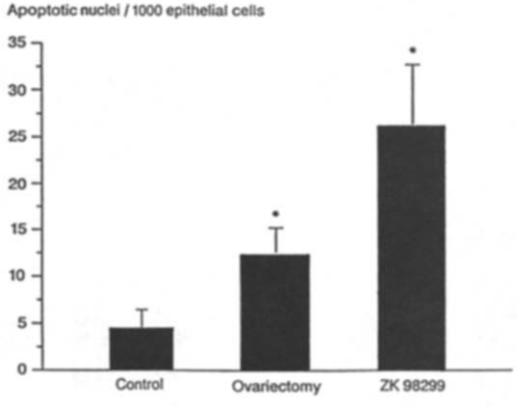


Fig. 3d. Morphometric analysis of the amount of apoptotic cell bodies in the MXT(+) mammary carcinomas.

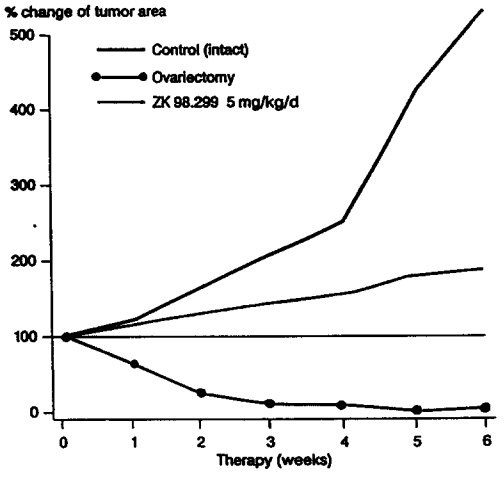
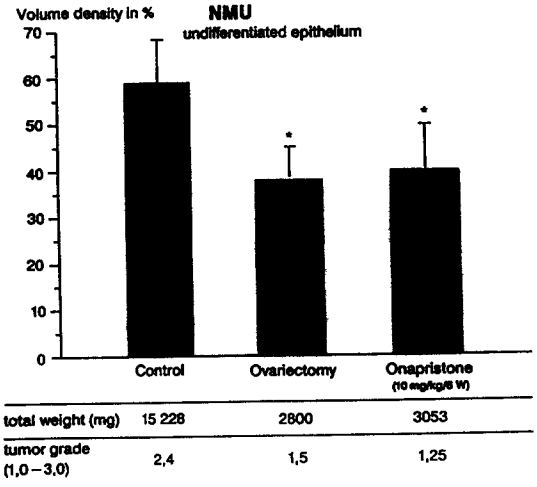


Fig. 4. Growth curves of the NMU-induced mammary carcinoma of the rat expressed as change of tumor area in percent.



	Control	Ovariectomy	Onapristone (10 mg/kg/6 w)
total weight (mg)	15 228	2800	3053
tumor grade (1,0–3,0)	2,4	1,5	1,25

Fig. 4a. Volume density of undifferentiated tumor epithelial cells in the NMU-induced mammary carcinoma.

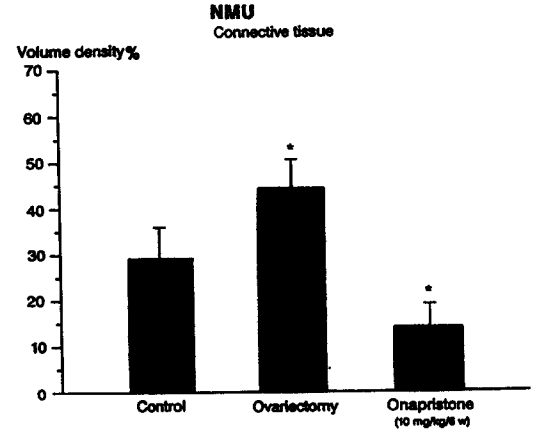


Fig. 4b. Volume density of connective tissue in the NMU-induced mammary carcinoma.

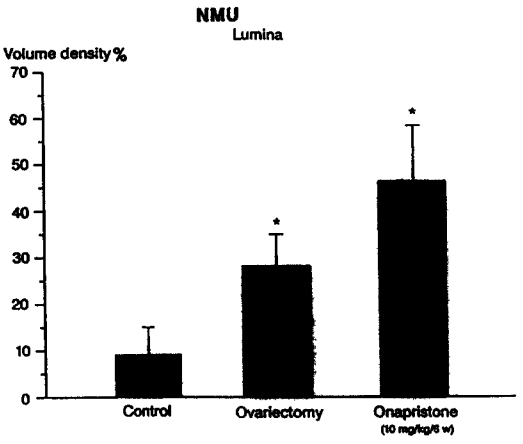


Fig. 4c. Volume density of glandular lumina in the NMU-induced mammary carcinoma.

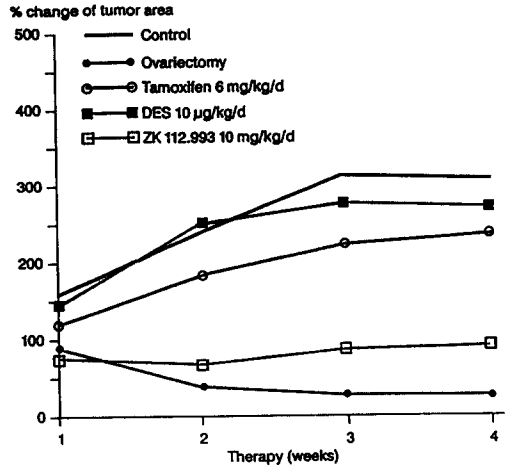
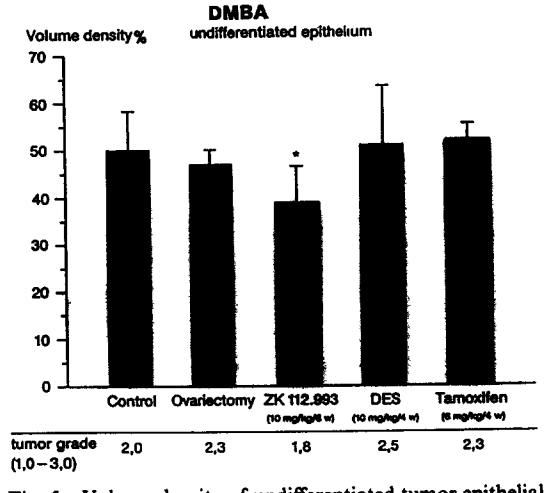


Fig. 5. Growth pattern in the DMBA-induced mammary carcinoma of the rat expressed as change of tumor area in percent.



	Control	Ovariectomy	ZK 112.993 (10 mg/kg/6 w)	DES (10 mg/kg/4 w)	Tamoxifen (6 mg/kg/4 w)
tumor grade (1,0–3,0)	2,0	2,3	1,8	2,5	2,3

Fig. 5a. Volume density of undifferentiated tumor epithelial cells in DMBA-induced tumors.

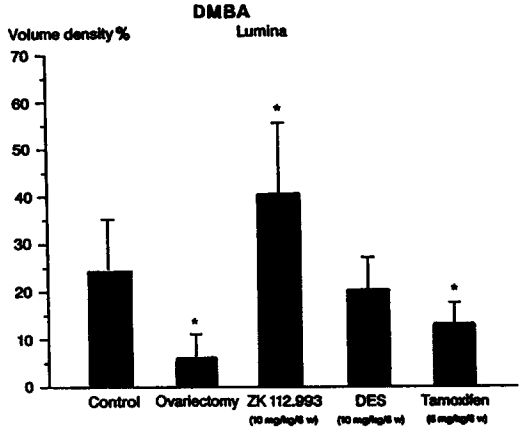


Fig. 5b. Volume density of glandular lumina in DMBA-induced tumors.

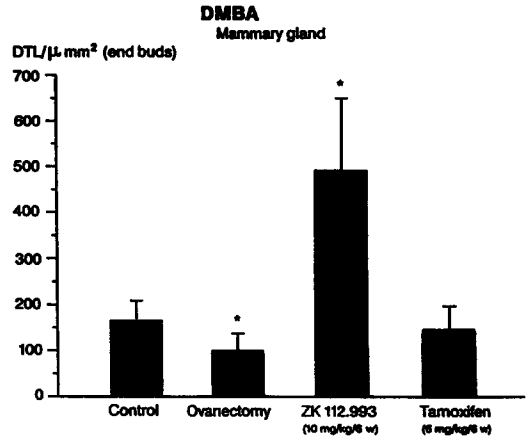


Fig. 5e. Volume density of tubulo–alveolar glandular buds of the mammary gland.

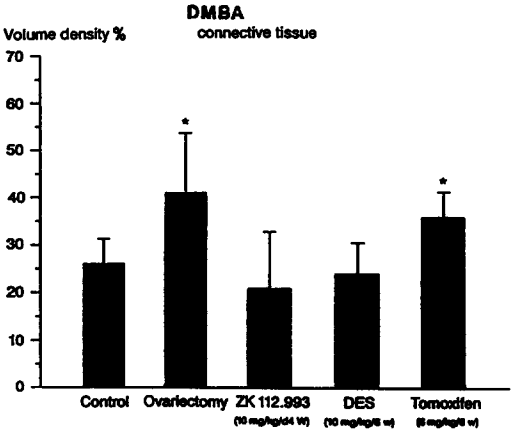


Fig. 5c. Volume density of connective tissue in DMBA-induced tumors.

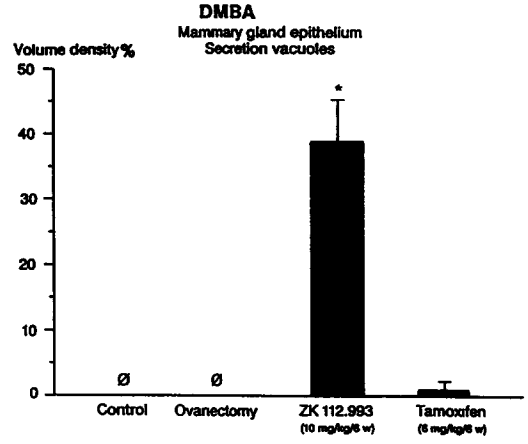


Fig. 5f. Volume density of secretion vacuoles of the mammary gland.

CONCLUSIONS

Our data indicate, that the described reaction pattern of these experimental mammary carcinomas after treatment with progesterone antagonists differs totally from the tumor inhibiting mechanism of ovariectomy, treatment with tamoxifen and high dose estrogen. The treatment with progesterone antagonists leads to terminal differentiation in the mammary carcinomas by induction of secretory active glandular formations with the disappearance of undifferentiated epithelial tumor cells.

The mammary glands of progesterone antagonist treated animals displayed the morphometrical features of differentiation with the appearance of secretory activity and—as a well accepted sign of terminal cell death—the appearance of apoptotic cell death. The staining pattern with UEA-I as an indicator of cell membrane bound fucosylation indicates a strong negative correlation with the degree of tumor differentiation: interestingly recent studies postulate a relationship between the expression of *neu* oncogene, the appearance of high molecular weight glycoproteins and some lectins [10–12].

In summary, our results underline the concept of a differentiation potential of progesterone antagonists on progesterone receptor positive mammary carcinomas, suggesting that such compounds offer an innovative and potent treatment strategy for hormone-dependent breast cancer.

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