Effects of Ionizing Irradiation on the Estradiol and Progesterone Receptors in Rat Mammary Tumors

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ABSTRACT

The determination of estradiol and progesterone receptor concentrations in mammary tumors is useful in predicting the hormone responsiveness. As this assay is carried out on tumor tissue which may have been subjected to radiotherapy, the possibility of an ionizing irradiation affecting the steroid receptor levels in neoplastic tissue should be taken into account. The steroid receptor concentrations are examined in dimethylbenz(a)anthracene-induced tumors of Sprague-Dawley rats. The estradiol and the progesterone receptor titers become reduced significantly after treatment with 20 Gray while an application with 7 Gray does not affect the titer values. After treatment of the tumor with 20 Gray, the steroid receptor concentrations decrease progressively, reaching a maximal reduction 20 to 30 days after exposure. This measured reduction in binding sites seems to be the result of a specific irradiation effect and not due to a possible increase in lytic enzyme levels in the regressing tumors. As radiation treatment affects the receptor concentrations, this should be kept in mind when interpreting the steroid receptor concentrations.

INTRODUCTION

Hormones remain valuable tools in the treatment of breast cancer complicated with metastases. However, the intrinsic risks of hormonal therapy, the relative low response rate (33%), and the time necessary to evaluate this treatment make a predictive test essential (20, 25). The determination of steroid receptors in malignant tissue can be considered such an effective predictive test which can be carried out before hormonal therapy is started. The hormone responsiveness becomes probable in about 50% of all cases when estrogen receptors are detected in the tumor (20, 34). Without detectable estrogen receptors, the possibility of hormone dependency falls to only 10% of all cases. The simultaneous and quantitative determination of estrogen (14, 19) and progesterone receptors (15, 32) possibly in conjunction with androgen (7, 31, 38, 41) and glucocorticoid receptor values (37) may even increase these prediction ratios. To obtain reproducible results, the steroid receptor assay should be carried out immediately after primary surgery. However, a possible preoperative flash irradiation or radiotherapy may affect the number of receptor-binding sites present in the tumor. In order to evaluate possible radiation effects, the steroid receptor concentrations are examined in irradiated mammary adenocarcinoma induced in rats by DMBA.

MATERIALS AND METHODS

Female Sprague-Dawley rats (Hannover strain × Hannover 91/D 3000; Zentral Institut für Verzuchtiere) 50 to 65 days of age received twice an intragastric dose of 10 mg DMBA (Fluka A. G. Buchs, Switzerland) dissolved in 0.5 ml of sesame oil with an interval of 24 hr (16, 17). For 1 week, the antibiotic Tylosin (Tylan-Eli Lilly and Co., Indianapolis, Ind.) (1 g/liter) was added to the drinking water to prevent pneumonia. The animals were fed on an ad libitum Purina chow diet. The first mammary tumors appeared 1 to 3 months after treatment. Only tumors measuring between 1 and 2.5 cm in diameter were used to avoid necrotic areas usually present in larger specimens. A series of animals was used as nonirradiated controls, a second series was treated with 7 Gray (1 Gray = 100 rads), and a third series received 20 Gray (cobalt teletherapy source). Radiotherapy on the tumors took place under general anesthesia with Nembutal (phenobarbital, 60 mg/kg). The tumors were measured for size immediately after irradiation and again when isolated from the animal. To determine estradiol receptor concentrations, the tissue was pulverized at −196°C and homogenized with 3 volumes of a TED buffer containing 10 mM Tris-HCl (Merck, Darmstadt, Germany), 1.5 mM EDTA (Calbiochem, Los Angeles, Calif.), and 0.5 mM 1,4-dithio-DL-threitol (Fluka A. G.) at pH 7.4. For the progesterone receptor assay, preparation took place in TED buffer containing 10% glycerol. After centrifugation at 105,000 x g (International Ultracentrifuge B60, Model SB 405 swinging bucket) for 70 min, the supernatant was used for the receptor determinations (22). The procedure in Cookes microtiter plates (V-shaped, Greiner), each with 8 x 12 cuvets of 0.2-ml volume, took place at 0 to 4°C. First, 5 µl of TED buffer were transferred to 8 cuvets in duplicate (Sorocore-Hamilton micropipets). Two cuvets received 5 µl of a 100-fold excess of unlabelled steroid dissolved in TED buffer or TED buffer containing 10% glycerol. These 2 cuvets as well as 2 others without unlabelled steroid received 50 µl of cytosol equally. Finally, each cuvet received 5 µl of radioactive labeled steroid: 17β-[2,4,6,7-3H]estradiol (specific activity, 94.0 Ci/mmol; New England Nuclear, Boston, Mass.) or 17α-[methyl-3H]promegestone (R5020; 17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione (17α-[methyl-3H]; specific activity, 87.0 Ci/mmol, New England Nuclear). For each assay, 6 concentrations, varying from 0.9 to 10 nmol of labeled estrogen and from 0.1 to 10 nmol of labeled R5020, were used. The titer plates were covered with parafilm and incubated overnight for 16 hr at 4°C in a vibrating microshaker. After the incubation, 2 cuvets not containing any cytosol received 100 µl DCC suspension [TED buffer-0.25% charcoal-0.025% dextran (Grade C; BDH Biochemicals, Poole, England)] to precipitate the free tracer. The measured radioactivity of the free tracer in the remaining supernatant never exceeded 2% of the initially added dose. The remaining tracer was considered a
DCC control. To 2 other cytosol-free cuvets, 100 μl of buffer were added, and the radioactivity measured here represented the total added tracer dose. The next 2 cuvets contained the totally bound tracer which corresponded to the radioactivity remaining after precipitation of the free steroid from the supernatant. The last 2 cuvets contained the aspecifically bound tracer. Cuvets 1, 2, 5, 6, 7, and 8 receiving 100 μl of DCC suspension were shaken for 10 min and centrifuged at 1000 \( \times g \) for 10 min (Size 2 international centrifuge, Model K, Rotor 240 with microtiter plate carrier). After centrifugation, 100 μl of a total of 160 μl in each cuvet were transferred to minicounting vials (Lumac Systems AG, Basel, Switzerland) to which 1 ml Aqua Luma scintillation liquid (Lumac Systems AG) was added. Counting took place in a liquid scintillation spectrometer (Rack Beta LKB, Wallac, OY, Turku, Finland). Finally, the steroid receptor concentrations were determined also in preparations obtained by mixing varying concentrations of extracts from control and irradiated tumors. After correction for background, DCC blank, and quenching, Scatchard analysis allowed the counts to be calculated via a linear regression curve into receptor concentrations and binding constants (33). The receptor concentrations were expressed in fmol/mg tissue or fmol/mg protein. Protein concentrations were measured according to the Folin phenol method (27). Statistical analysis was carried out according to the variant analysis and controlled by the Mann-Whitney U test. For microscopical analysis, the tumor tissue was fixed in Bouin-Hollande solution and stained with a routine hematoxylin and eosin coloration.

RESULTS

Morphological Analysis. All the glandular tumors examined presented a lobular or nodular architecture. Differentiation of the various tumor structures differed only slightly in function of the cell density. Some areas were more glandular while others presented a more papillary growth. The tumor cells had a rather quiet cytological aspect without any obvious nuclear anomalies although multiple mitoses were present. The nuclei were oval or rounded with a fine chromatin structure, most with one or 2 small nucleoli. The cytoplasm was slightly eosinophilic. The nucleocytoplasmic index seemed rather high. All these DMBA-induced tumors were highly radiosensitive. On the fourth day after radiotherapy with 20 Gray, the average tumor diameters were reduced by 50%, reaching a maximal reduction around the 20th to 30th days. Tumor growth reappeared about 30 days after treatment and reached again 50% of the initial size around Day 60. Nonirradiated control tumors continued to grow at an accelerated rate (Chart 1). The morphological characteristics of the tumors irradiated with 7 or 20 Gray were almost identical to those of the control tumors, and only minor changes in the differentiation were observed. Although histological modifications due to irradiations seemed negligible, macroscopic analysis showed the irradiated tumors to contain greater areas of cystic necrosis. Necrotic tissue was carefully avoided in the samples used for the receptor determination.

Irradiation Effects on Receptor Concentrations. The estrogen receptor concentration in DMBA-induced tumors amounted to 129.0 ± 24.5 fmol/mg protein (mean of 30 animals ± S.E.) while the concentrations in tumors treated with 20 Gray examined 5 days after irradiation were reduced to 47.8 ± 13.5 fmol/mg protein (mean of 30 animals ± S.E.). After radiotherapy with 7 Gray, no significant differences were observed. A progressive reduction of estrogen receptor and progesterone receptor concentrations to 50% of the initial values was observed around the fifth day after radiotherapy. After irradiation, the initial reduction in steroid receptor concentrations followed the pattern of tumor regression. The estradiol receptor concentrations reached a low level around 30 days after irradiation which did not increase even after renewed tumor growth (Chart 3). The progesterone receptor concentrations were equally reduced with the lowest number of binding...
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Chart 2. Steroid receptor concentration in function of radiation therapy. The steroid receptor concentrations in DMBA-induced mammary tumors are compared 5 days after radiotherapy. A, values obtained with nonirradiated tumors; B, tumors irradiated with 20 Gray. The receptor concentrations are expressed as fmol/mg tissue. Point, steroid concentration in one tumor each from a different animal. For each population, the average receptor concentration is given with the S.E.

Chart 3. Modifications in 17β-estradiol receptor concentrations in function of a 20-Gray irradiation. •, 17β-estradiol receptor concentrations determined before and at different times after an irradiation with 20 Gray. Point, mean of 8 experiments; bars, S.E. O, receptor concentrations in tumors of nonirradiated control animals in function of time.

Chart 4. Modifications in progesterone receptor concentrations after 20-Gray treatment. •, progesterone receptor concentrations determined in tumors before and on different days after treatment. Point, mean of 8 experiments; bars, S.E. O, receptor concentrations in tumors of nonirradiated control animals in function of time.

Chart 5. Comparison between estradiol receptor values (A) and the progesterone receptor values (B) in nonirradiated controls (O) and in tumors irradiated with 20 Gray which are in regression (a), in an apparent state of no growth (i>, or in a regrowth phase (c). n, numbers of tumors examined; bars, S.E.

sites occurring almost at the same time as that observed for the estradiol receptor. However, as tumor regrowth progressed, the progesterone receptor levels seemed to recover, although statistically still insignificant to be interpreted (Chart 4). Estradiol as well as progesterone receptor levels in nonirradiated control tumors remained almost constant over the whole experimental period and did not differ significantly from the initial control values in spite of the continuous growth of the control tumors. The estradiol and progesterone receptor concentrations did not differ significantly when comparing different groups of irradiated tumors, either in regression, in an apparent steady state of no growth, or in a regrowth phase (Chart 5). In mixing experiments, control tumor extracts were combined in varying concentrations with extracts from tumors 5 days previously irradiated with 20 Gray. The measured estradiol and progesterone receptor levels of the mixtures corresponded to the theoretically calculated values (Chart 6).

DISCUSSION

DMBA-induced mammary tumors are characterized by a relatively benign histological aspect with virtually no metastases. As in human breast cancer, the hormone dependency is related to the steroid receptor concentrations. These experimental tumors are also sensitive to radiotherapy. Although the results obtained from experimental animal tumors are not to be extrapolated to the human variety, certain similarities appear to exist (9, 18, 22, 28, 30, 31, 36). Human breast tumors may receive a preoperative irradiation to reduce the tumor volume and possibly to avoid metastases of tumor cells during subsequent mastectomy (5). Receptor concentrations in the removed tumor tissue may predict to a great extent the hormone dependency of possible metastases. Thus, the possible effects of a preliminary irradiation on the steroid receptor levels are important to be recognized. The application of 20 Gray, usually administered as a preoperative radiotherapy, results in a marked regression of the tumor size. Morphological changes specifically due to an ionizing irradiation are not detectable, and the tumor regression seems to progress uniformly without the selective disappearance of any specific structures (29, 35, 42). The reduction of estradiol and progesterone receptor concentrations after 20 Gray takes place simultaneously with the tumor regression. These irradiation effects are not observed when MCF-7 tissue culture cells are analyzed, which might be a reflection of the differences between in vivo and in vitro systems as observed by the differences in receptor metabolism between MCF-7 cells and DMBA-induced tumor cells (5, 8). In human breast cancer, however, a reduction in steroid receptor concentrations after radiotherapy has been reported (4). After irradiation, an increased release of lytic enzymes might affect the receptor proteins and thus artificially reduce the steroid
receptor-binding sites. In a mixing experiment, control tumor extracts are combined in varying proportions with extracts from tumors 5 days previously treated with 20 Gray. The concentrations of estradiol and progesterone receptors obtained correspond to the theoretically calculated values. This seems to indicate that the main effects of irradiation on the steroid receptor-binding sites cannot be attributed to an artefact due to interference by excess release of proteolytic enzymes. Furthermore, as the concentrations of receptor-binding sites do not differ significantly in the various irradiated tumors whether in involution or in a regrowth phase, the reductions in receptor concentration may be interpreted to be a direct effect of ionizing irradiation rather than provoked by interfering factors (10–13, 23, 24, 40). Endocrinological manipulations to affect these receptor levels can also be eliminated as the receptor levels of the uterus carried out simultaneously are identical in controls and irradiated animals (22, 26). A possible effect of endogenous hormone secretion from gonadal origin might still be considered responsible for a reduction in the free cytoplasmic steroid receptor levels. This, however, is most improbable as no differences are found between the reductions in estradiol and progesterone receptor concentrations after irradiation of tumors above and below the diaphragm. Tumor regression after castration indeed presents a completely different pattern and morphological characteristics (1–3, 6, 21, 26, 31). With the reduction in estrogen concentration after castration, the estradiol receptor concentrations are expected to remain initially constant or slightly reduced, accompanied by a drastic reduction of the progesterone receptors (2, 3, 22). The simultaneous reduction in steroid receptor concentrations and tumor size could indicate that the receptor-containing cells (target cells) might be more radiosensitive. Possibly, this reduction in steroid receptors might be the result of radiation damage selectively inflicted on nucleic acids which essentially condition receptor metabolism and cell growth.

REFERENCES

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