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IN VITRO SYNERGISM BETWEEN ESTROGENS AND CYTOTOXIC AGENTS

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Summary—Two lines of research intending to achieve synergism between cytotoxic agents and estrogens for breast cancer treatment are pursued in our laboratory.

1. According to a screening procedure we select cytotoxic-linked estrogens which bind to estrogen receptors and thereby would be specifically concentrated into the tumor cells. A mesylate derivative of estrone has emerged from our investigations. This compound displays a very strong binding affinity for the receptors and inhibits the growth of the receptor-positive MCF-7 breast cancer cell line. The lack of growth inhibition in the receptor-negative line Evsa-T indicates that it is devoid of major non-specific cytotoxicity.

2. We attempt to enhance the vulnerability of the tumor cells by producing an estrogen-induced modification of their chromatin. The *in vitro* exposure of isolated uterine nuclei to uterine cytosol preincubated with estradiol increases their ability to bind [^{3}H]actinomycin D. Identical results are obtained with MXT mouse mammary tumors. Experiments are in progress to settle whether these changes are associated with enhanced cell killing.

INTRODUCTION

In recent years, our knowledge of the mechanism by which estrogenic hormones act on mammary tumor cells has increased greatly. It is now well-established that estrogens bind to the cytoplasmic estrogen receptors (ER) as they do in the uterine cells. The resulting complexes are then translocated to the cell nucleus where they accumulate and produce structural modifications of the chromatin. These events are the preliminary steps leading to RNA and protein synthesis.

One may legitimately hope that a rational use of this mechanism may form the basis for new synergistic associations of estrogens and cytotoxic agents for the treatment of breast cancer. In this regard, two main lines of research are pursued in our laboratories:

(1) to produce cytotoxic-linked estrogens which bind to ER and are selectively concentrated into the tumor cells;

(2) to increase the vulnerability of the tumor cells to intercalating agents by producing an estrogen-induced modification of the chromatin.

As yet, we have limited our investigations to *in vitro* studies for evaluating the potential value of this receptor-mediated chemotherapy. The present paper overviews our results and defines our projects.

CYTOTOXIC-LINKED ESTROGENS

The use of steroid hormones as specific carriers of cytotoxic agents for the treatment of hormone-dependent carcinoma is not a new task: in the 1960s a large number of alkylating steroids were synthesized [1, 2]. The absence of knowledge about the interaction between the steroids and their receptors led to the production of drugs devoid of binding affinity and specific therapeutic activity. With regard to estrogens, clinical trials showed the absence of therapeutic value of *estracyt* and *estradiol mustard* for the treatment of advanced breast cancer [3–5].

Our studies demonstrated the absence of binding affinity of estracyt and estradiol mustard for ER [6, 7]. In these two compounds, the 3-phenolic group of estradiol is substituted by the alkylating function. In view of the major importance of this group in the binding of the estrogens to ER, it has been proposed that such a substitution is mainly responsible for their lack of binding affinity. Alkylating derivatives of estradiol and estrone with a free phenolic group were therefore synthetized. Similarly, non-steroidal estrogens (diethylstilbestrol, hexestrol) were substituted elsewhere than positions 4 and 4′ since the corresponding phenolic functions are assumed to recognize the receptors sites interacting with the 3- and 17-hydroxyl functions of estradiol.

Several drugs of this new generation were tested in our laboratory. In the classical competitive test of the binding of [^{3}H]estradiol to ER [8], most compounds displayed a weak but significant binding affinity ($\sim$1% of E_2). One of them, a mesylate derivative of estrone* (formula given in Fig. 1), showed a very strong binding affinity roughly similar to that of estrone. This property was most probably due to an irreversible binding of the compound with the receptors. Moreover, it inhibited the growth of the MCF-7 (ER-positive) breast cancer cell line at concentrations as low as the strongest antiestrogens hydroxy-

*Compound produced by Dr R. L. Morgan, Louisiana State University, New Orleans, La.

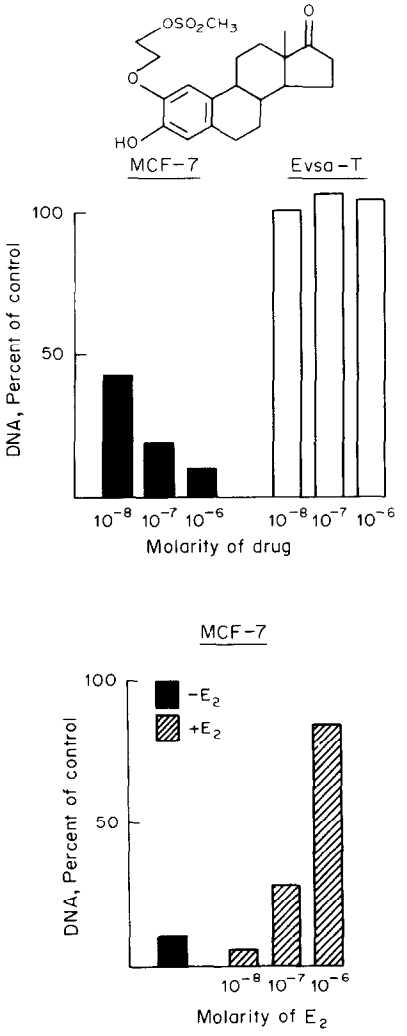

Fig. 1. Cytotoxic effect of a mesylate derivative of estrone on MCF-7 and Evsa-T cell growth. The upper graph shows that the compound inhibits the growth of the MCF-7 cells while it has no effect on the Evsa-T cells. The lower graph shows that the inhibition of the compound is suppressed by estradiol. For further details see Ref. [9].

tamoxifen and CI-628 M (Fig. 1) [9]. This inhibition was suppressed by estradiol, indicating its specificity [9, 10]. Remarkably, hydroxylated and methoxylated analogs of estrone (2-OH, 2-OCH_3) did not inhibit growth, suggesting that the cytotoxicity of the compound was associated with its mesylate residue. That no growth inhibition occurred in the Evsa-T (ER-negative) breast cancer cell line also indicated that the compound was devoid of major non-specific cytotoxicity.

The mode of action of this mesylate derivative of estrone is unknown. However, its irreversible binding with ER highly suggests that it may act as a "suicide inhibitor". With regard to its potential therapeutic interest, our data are obviously too scarce to ascertain an important role. *In vivo* experiments are planned to further evaluate its antitumor activity as well as its endocrinological properties. Efforts are also presently under way to link reagents for proteins on estrogens and antiestrogens to produce other suicide inhibitors.

ESTROGEN-INDUCED INCREASES IN TUMOR CELL VULNERABILITY TO INTERCALATING DRUGS

Nuclear transfer of the estrogen–receptor complexes produces an increase in RNA synthesis, most probably by derepressing genomic sites. Some years ago, we investigated this phenomenon by the autoradiographic method of [^{3}H]actinomycin D ([^{3}H]ACT-D) labeling introduced by Brachet and Ficq[11] for evaluating derepression processes. Uterine nuclei were incubated with uterine cytosol and subsequently smeared on histological slides for the [^{3}H]ACT-D labeling [12]. Under these experimental conditions, we found that a preincubation of the cytosol with estradiol, allowing the nuclear transfer of the receptors, produced a significant increase of [^{3}H]ACT-D binding. This phenomenom did not occur under conditions giving no increase in RNA synthesis, indicating the high specificity of the assay. It is noteworthy that our observations were biochemically confirmed by Mainwaring and Jones[13] on isolated chromatin from prostatic tissue.

Pursuing our investigations, we recently showed that uterine cytosol preincubated with hydroxytamoxifen did not produce any increase or decrease of [^{3}H]ACT-D binding. On the contrary, the antiestrogen counteracted the increase of binding induced by estradiol, which is consistent with its known antagonistic activity [14].

All these data led us to investigate mammary carcinomas. Hormone-sensitive MXT mouse mammary tumors [14, 15] were used in a preliminary study. A significant increase of [^{3}H]ACT-D binding occurred in isolated nuclei exposed to cytosol preincubated with estradiol. Again, the increase was not found under various experimental conditions giving no nuclear transfer of the receptors, i.e. cytosol alone, estradiol alone, cytosol in which estradiol was added at the time of the exposure to the nuclei (Fig. 2).

These results led us to hypothesize that the increase in [^{3}H]ACT-D labeling induced by estradiol might be associated with a higher vulnerability of cells to intercalating agents and possibly to other cytotoxic drugs interacting with DNA. Experiments are presently being conducted to evaluate this hypothesis. In a first step, [^{3}H]daunomycin ([^{3}H]DNR) was tested to evaluate the repeatability of the results. Unfortunately, a high level of unspecific binding was found (binding on membrane) indicating that experimental conditions for [^{3}H]ACT-D were nonappropriate for [^{3}H]DNR. Modifications of these conditions appear, therefore, necessary to pursue our objectives. In the following step, we plan to culture MCF-7 cells with [^{3}H]ACT-D and [^{3}H]DNR in the absence or presence of estradiol to investigate the

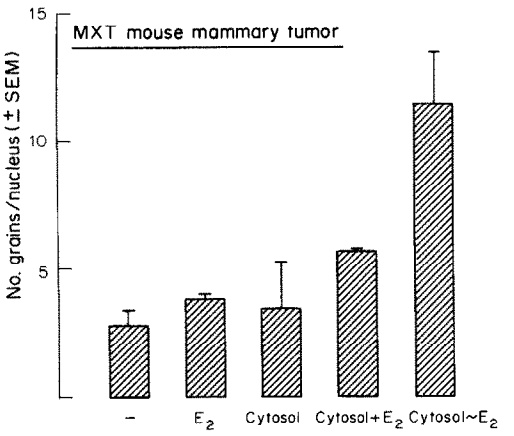

Fig. 2. Binding of [^{3}H]ACT-D to purified nuclei maintained in buffer (—) or exposed to 10^{-8} M estradiol (E_2), cytosol (cytosol), cytosol + 10^{-8} M estradiol (cytosol + E_2) and cytosol preincubated with 10^{-8} M estradiol (cytosol ~ E_2). Variance analysis of the data shows that [^{3}H]ACT-D binding is significantly higher in nuclei exposed to preincubated cytosol. No significant difference occurs between the other experimental conditions. For further details see Refs [14, 15].

occurrence of our observations in living cells. These investigations are extremely important since all the present experiments were carried out under non-physiological conditions (fixed material, isolated chromatin). The potential improvement of antitumor activity of intercalating agents induced by the concomitant or shortly preceding administration of estradiol, of course, will also be assessed.

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