Antiestrogen Action of 2-Hydroxyestrone on MCF-7 Human Breast Cancer Cells*

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The estrogen responsive human breast cancer MCF-7 cell culture was examined for its response to 2-hydroxyestrone, a principal metabolite of estradiol. Addition of 2-hydroxyestrone to the cell cultures in concentration of 10^-10^-4 M had no effect on cell growth and proliferation because of rapid O-methylation of the catechol estrogen by catechol O-methyltransferase which is highly active in these cells. In the presence of quinalizarin, a potent catechol O-methyltransferase inhibitor which reduces the O-methylation of the steroid, 10^-7 M and 10^-8 M 2-hydroxyestrone markedly suppresses the growth and proliferation of the cells. The tumor cell growth-inhibitory action of the catechol estrogen was neutralized by the presence of 10^-8 M estradiol. The catechol estrogen inhibition of cell growth is not observed in the estrogen receptor-negative human breast cancer cell lines MDA-MB-231 and MDA-MB-330 providing evidence that the inhibition is specific and is estrogen receptor-mediated. In contrast, the 16a-hydroxylated metabolites of estradiol, estriol and 16a-hydroxyestrone, are effective stimulators of MCF-7 cell proliferation with the latter exhibiting potency in excess of that expected from its estrogen receptor affinity. The present results represent the first observation of a specific receptor-mediated antiestrogenic action of 2-hydroxyestrone and suggest that the physiological regulation of the agonist activity of the primary estrogen may involve in situ generation of catechol estrogen.

The catechol estrogens are quantitatively the most important metabolic products of the female sex hormone estradiol (1). Although their exact physiological role in the mediation and/or modification of the hormonal actions of estrogen is still the subject of much study, they have already been shown to exert numerous biological effects in both peripheral as well as central estrogen target tissues. These effects can be characterized as reflecting either estrogen agonist or antagonist actions with the most prolific catechol estrogen, 2-hydroxyestrone, exhibiting minimal agonist activity but providing evidence of estrogen antagonist action in neuroendocrine events (2-4). The mechanisms underlying the action of the catechol estrogen are not clearly understood, although data on their interaction with the estrogen cytosolic receptor (5, 6) and its subsequent translocation into the nucleus (7, 8) indicate that at least some of their actions may involve competition with the primary estrogen for the receptor. The catechol estrogen may also act via their interaction with the catecholaminergic system. They have been reported to bind specifically to dopamine and α1-adrenergic receptors in the brain and pituitary (9, 10). They inhibit tyrosine hydroxylase, the rate-limiting enzyme of catecholamine biosynthesis (11, 12) and can thus decrease catecholamine content. Conversely they are highly effective competitive inhibitors of the metabolism of catecholamines by catechol O-methyltransferase and would thus decrease the turnover time of these biogenic amines (13, 14). Indeed, it is now apparent that catechol estrogen administration in vivo are rapidly metabolized by the action of tissue and erythrocyte catechol O-methyltransferase resulting in very high metabolic clearance rates for these compounds (15-17). This feature of their biology makes it difficult to define their intrinsic biological properties by in vivo administration.

In the present study we have used the hormone-dependent human breast cancer cell line MCF-7 in tissue culture as a system for the study of the biological effects of 2-hydroxyestrone, under conditions where its further metabolism could be controlled, and its activity in peripheral estrogen target sites be evaluated. Our results indicate that the catechol estrogen behaves as an antiestrogen in suppressing the tumor cell proliferation both under control and estradiol stimulatory conditions. This activity of the catechol estrogen becomes apparent in the presence of the inhibitors of catechol O-methyltransferase an enzyme whose activity is greatly increased in this tumor tissue.

**EXPERIMENTAL PROCEDURES**

**Materials**—Human breast cancer cell lines MCF-7, MDA-MB-231, and MDA-MB-330 were obtained from the Breast Cancer Human Cell Culture Bank of the National Cancer Institute (maintained by the EG & G Mason Research Institute, Worcester, MA). 2-[(6,7-3H)hydroxyestrone (specific activity, 40-60 Ci/mmol), purchased from New England Nuclear, was purified by thick layer chromatography as described (18) to ensure the absence of any contamination by other steroids, particularly estradiol. Estradiol and estriol were obtained from Steroids, Inc. (Wilton, NH). 16a-hydroxyestrone from Sigma, and quinalizarin from Aldrich Chemical Co. All steroids were checked for purity by chromatographic and spectral criteria, and were purified to homogeneity as required. RPMI 1640 medium, fetal bovine serum, penicillin-streptomycin suspension (10,000 units and 10,000 µg, respectively/ml), mycostatin suspension for purity by chromatographic and spectral criteria, and were purified to homeogeneity as required. RPMI 1640 medium, fetal bovine serum, penicillin-streptomycin suspension (10,000 units and 10,000 µg, respectively/ml), mycostatin suspension (10,000 units/ml), trypsin-EDTA (1X), Eagle's balanced salt solution, and Eagle's balanced salt solution without Mg2+ and Ca2+ were obtained from Gibco Labs (Grand Island, NY). Sterilization filters (0.45 μ and 250 ml or 500 ml capacity) were purchased from Netherlands Co. (Rockefeller, NY).

**Cell Culture**—The MCF-7 cells were subcultured in T-75 flasks by daily changes of the "passage" medium (50 ml) consisting of RPMI 1640 supplemented with fetal bovine serum (10%, v/v), which were maintained in a humidified chamber at 37 °C with 5% CO2 and 95% air. The cells were harvested at confluency by trypsinization and suspended at a cell density of 200,000 to 300,000 cells/ml in a small volume of the growth medium to be used.

In a typical experiment in which the effects of hormones on the...
cell growth were to be examined, aliquots of the cell suspension containing about 20,000 to 30,000 cells were repitically plated in dishes (60 mm in diameter) containing 3 ml of the growth medium consisting of RPMI 1640 supplemented with charcoal-stripped fetal bovine serum (5%, v/v) and of penicillin-streptomycin (10 units and 10 µg, respectively/ml of medium) and mycostatin (2.5 units/ml) and incubated at 37°C in the humidified chamber. On day 1 when the cells adhered firmly to the bottom surface of the dishes, the medium was aspirated off and the cells then challenged by hormones; groups of replicate dishes received 3 ml of fresh growth medium containing a hormone at the indicated concentrations. Another group of replicate dishes which received only the vehicle served as the nonhormone-treated controls. All dishes, unless otherwise stated, also received quinalizarin (dissolved in dimethyl sulfoxide), a potent inhibitor of catechol O-methyltransferase (19), at a concentration of 10-8 M, a concentration which does not exert any effect on cell growth but is effective in inhibiting the enzyme. Each group received a change of the fresh growth medium (3 ml) every 24 h unless otherwise noted, at which time the hormone-treated group received the hormone at the indicated levels and the control group the vehicle. At various time intervals, i.e. 24 h, triplicate dishes were withdrawn for cell counting; after removal of the medium by aspiration, the adhered cells were washed twice with 1 ml of Eagle’s balanced salt solution without Ca2+ and Mg2+ and dislodged from the dish by trypsinization involving the incubation with 0.5 ml of trypsin-EDTA solution (1X) for 5 min at 37°C, after which the protease reaction was stopped by adding 2.5 ml of a “stop” medium consisting of RPMI 1640 and 5% fetal bovine serum. The cells were then collected by centrifugation with subsequent suspension in the medium. An aliquot of the suspension was subjected to cell counting using a hematocytometer. In the present study we focused on cell proliferation as an index of estrogen response since other endpoints such as [3H]thymidine incorporation are subject to complex variables (20, 21).

The estrogen-independent human breast cancer cell lines MDA-MB-231 and MDA-MB-330 were cultured in a manner identical with that described above for MCF-7. Metabolism of 2-Hydroxyestrone and Identification of Its Metabolic Products—150 pmol of 2-[6,7-3H]hydroxyestrone (containing about 1.32 million dpm) were added to groups of replicate MCF-7 cells on day 6 when they were growing exponentially (see Fig. 1), and then exposed for the various time intervals indicated, after which all of the medium (3 ml) was collected for analysis of the metabolites. Then 25 µg of 2-hydroxyestrone (dissolved in AMA solution consisting of 20% ascorbic acid, 78% methanol, and 2% acetic acid) was added as a carrier. The medium was extracted with 3 ml of ethyl acetate by mixing vigorously in a vortex for 1 min, and collecting the upper organic layer after centrifugation at 2500 g for 10 min and evaporating to dryness. The residue was then taken up in 100 µl of methanol and received 10 µg of 2-methoxyestrone as a standard, after which an aliquot of the methanolic solution was subjected to thin layer chromatography on Silica Gel G in a solvent system consisting of ethanol/chloroform = 5/95 (v/v). 2-Hydroxyestrone and 2-methoxyestrone had Rf values of 0.51 and 0.76, respectively, in this solvent system. The isomeric 3-methyl ether of 2-hydroxyestrone another possible product of O-methylation was coincident with 2-methoxyestrone. The resulting thin layer chromatographic plate was scanned by Packard Model 385 Radiocromatogram Scanner.

RESULTS

Rapid Metabolism of 2-Hydroxyestrone by O-Methylation—Our initial attempt to demonstrate the effect of 2-hydroxyestrone on the tumor cell growth and proliferation of a hormone-dependent human breast cancer cell line, MCF-7, in cell culture, met with a lack of response. Analysis of the metabolites of the catechol estrogen, however, revealed that the failure to observe any effect was due to rapid metabolism of the steroid via O-methylation by catechol O-methyltransferase which was highly active in these cells. As shown in Fig. 1, 2-hydroxyestrone, when presented at a concentration of 10-7 M to the 600,000 cells growing exponentially in RPMI 1640 medium supplemented with 5% calf bovine serum stripped of endogenous estrogen, was rapidly metabolized to O-methylated products as analyzed by thin layer chromatography. The metabolism is essentially complete in 1–2 h with 30 to 40% of the total dose being O-methylated and remaining unchanged subsequently for up to 48 h. The remainder of the dose is retained intracellularly; the nature of the cell-retained material was not investigated. It should be noted that 2-hydroxyestrone appears to undergo a two-stage metabolism in this system; an early phase conversion to unidentified polar metabolites (Fig. 1) and a subsequent biotransformation to 2-methoxyestrone. In any event no intact 2-hydroxyestrone is

FIG. 1. Rapid metabolism of 2-hydroxyestrone (2-OHE) in the human breast cancer cell line MCF-7 and identification of its metabolites by thin layer chromatography. The cells were grown as described under “Experimental Procedures” except that the medium consisted of RPMI 1640 containing 5% fetal bovine serum and 2-[6,7-3H]hydroxyestrone was added to the cells at a final concentration of 5 x 10-8 M on day 6 when the cells were growing exponentially. At various time intervals indicated, the medium was withdrawn, processed, and analyzed for the metabolites by thin layer chromatography as described under “Experimental Procedures.” 2MeOE, 2-methoxyestrone.
becomes apparent in the presence of the potent catechol stimulator and the catechol O-methyltransferase inhibitor control cell groups received the medium containing the vehicle alone and the inhibitor alone, respectively. Media changes were made every other day (on days 3 and 5). At various time intervals, i.e. 24 h, triplicate dishes in each group were removed and subjected to cell counting as described under "Experimental Procedures." The points represent mean of triplicate samples. Hormone challenges were: , 2-hydroxyestrone, 10^{-7} M; ---, none (the vehicle alone); ×--×, none (quinalizarin, 10^{-8} M); ---, 10^{-7} M estradiol; and Δ--Δ, 10^{-7} M estradiol.

**FIG. 3 (center).** Reversal of the estrogen receptor-induced growth inhibition of MCF-7 cells by the primary estrogen estradiol. Groups of replicate dishes containing an equal number of the cells, plated on day 0 under the conditions described under "Experimental Procedures," were challenged by the catechol estrogen alone, by estradiol alone, or by the catechol estrogen and estradiol simultaneously in combination at the indicated concentrations on day 1 as indicated by the arrow. Control groups received no hormone but the vehicle and all groups also received quinalizarin at a concentration of 10^{-6} M. Media were changed every other day (on days 3 and 5) when each group received its respective hormones singly or in combination, together with the catechol O-methyltransferase inhibitor. At the indicated time intervals triplicate dishes were withdrawn and subjected to cell counting as described under "Experimental Procedures." Hormone challenges were: , 10^{-7} M 2-hydroxyestrone; ---, none; ---, 10^{-7} M 2-hydroxyestrone plus 10^{-11} M estradiol; ---, 10^{-11} M estradiol alone; Δ--Δ, 10^{-7} M 2-hydroxyestrone plus 10^{-4} M estradiol; and Δ--Δ, 10^{-4} M estradiol alone.

**FIG. 4 (right).** Irreversibility of the action of the catechol estrogen on the cell growth and proliferation of MCF-7 cells. Groups of replicate dishes were exposed daily to 2-hydroxyestrone (2OHE1) at the concentration of 10^{-7} M. A group of control cells received the vehicle alone. The catechol estrogen-treated groups were then challenged by estradiol (E2) on day 4 (as indicated by the arrow) at either 10^{-11} or 10^{-8} M, both concentrations adequate to normally stimulate the cell growth and proliferation of the estrogen-responsive tumor cells (Fig. 3). Quinalizarin was present at a concentration of 10^{-6} M in all dishes. Media were changed daily. At various time intervals estradiol treatment on day 4, triplicate dishes were removed for cell counting as described under "Experimental Procedures." ---, hormone-ununtreated control cells; ---, cells treated with 2-hydroxyestrone (10^{-7} M) throughout the experiment; ---, cells treated with 2-hydroxyestrone (10^{-7} M) until day 4 and then simultaneously challenged to both the catechol estrogen (10^{-7} M) and estradiol (10^{-11} M) thereafter in an attempt to rescue the cells; and Δ--Δ, cells treated with 2-hydroxyestrone (10^{-7} M) until day 4 and then simultaneously challenged to both the catechol estrogen (10^{-7} M) and estradiol (10^{-8} M) thereafter in an attempt to rescue the cells.

discernible in the medium approximately 1 h after its introduction.

**Antiestrogenic Activity of 2-Hydroxyestrone in the Presence of Catechol O-Methyltransferase Inhibitors.**—The response of the human breast cancer cell MCF-7 to the catechol estrogen becomes apparent in the presence of the potent catechol O-methyltransferase inhibitor, quinalizarin (19), at a concentration of 10^{-6} M which does not cause cytotoxicity to the cells but does inhibit the enzyme (Fig. 2). Under these conditions, 2-hydroxyestrone at a concentration of 10^{-6} M or 10^{-7} M effectively inhibited cell proliferation of the MCF-7 cells. Under identical conditions estradiol at as little as 10^{-11} M stimulated the tumor cell growth approximately 2-fold over the rate of unstimulated control cells, with a maximal stimulation occurring at a concentration in the range of 10^{-6} to 10^{-8} M.

**Reversal by Estradiol of 2-Hydroxyestrone-induced Growth Inhibition.**—The tumor cell growth inhibition elicited by the 10^{-7} M 2-hydroxyestrone in the presence of the catechol O-methyltransferase inhibition (i.e. quinalizarin at 10^{-6} M) was overcome by the simultaneous challenge on day 1 by estradiol at a concentration in the range of 10^{-11} to 10^{-9} M (Fig. 3), suggesting that the 2-hydroxyestrone and the primary estrogen compete with each other for the cellular site(s) of action which is presumably the estrogen receptor. This result is consistent with the notion that the tumor growth-inhibitory action of 2-hydroxyestrone is mediated by the estrogen receptor.

**Inability of Estradiol to Rescue the 2-Hydroxyestrone-inhibited MCF-7 Cells.**—In contrast to the result of the experiment in which the simultaneous challenge on day 1 of the primary estrogen and the 2-hydroxyestrone results in the reversal of the catechol estrogen inhibition, the addition of the former (at a concentration of 10^{-10} or 10^{-9} M) on day 4 under identical condition failed to rescue the catechol estrogen-inhibited cells (Fig. 4), suggesting that in the absence of estradiol during the
early days of cell growth the catechol estrogen effects an irreversible alteration of the estrogen-responsiveness of the MCF-7 cells. The irreversible action of the catechol estrogen may also involve estrogen receptor-linked events.

Effects of 2-Hydroxyestrone on the Tumor Cell Growth of Estrogen Receptor-negative Human Breast Cancer Cells—An additional line of evidence supporting the notion that the tumor cell growth-inhibitory action of 2-hydroxyestrone is brought about by estrogen receptor-mediated events stems from the results of the experiments (Fig. 5) indicating that estrogen receptor-negative human breast cancer cell lines, such as MDA-MB-231 and MDA-MB-330, grown under conditions similar to those for the MCF-7 cells, do not exhibit any growth-inhibitory response to 2-hydroxyestrone at a concentration of 10^{-7} M (Fig. 5b) in the presence of catechol O-methyltransferase inhibitors. Under identical conditions the estrogen receptor-positive MCF-7 cell exhibited the growth-inhibitory response to 2-hydroxyestrone as well as the growth-stimulatory response to estradiol (Fig. 5a). Both of the estrogen receptor-negative cancer cell lines did not respond to estradiol even at as high as 10^{-8} M (Fig. 5b), confirming their estrogen-unresponsiveness of both cell lines. These results, taken together, suggest that the action of 2-hydroxyestrone on the MCF-7 cells is mediated by the estrogen receptor-related events.

Effects of 16α-Hydroxy Metabolites of Estradiol on the Cell Growth of MCF-7—We examined 16α-hydroxyestrone and estradiol, the estradiol metabolites formed in the 16α-hydroxylative pathway, for their ability to affect the growth of the MCF-7 cells. The results, shown in Fig. 6, indicate that these metabolites, unlike the 2-hydroxylated derivatives, promote the cell proliferation of the breast cancer cells at concentra-

Fig. 5. Effects of the catechol estrogen, 2-hydroxyestrone, on the tumor cell growth of estrogen receptor-positive and estrogen receptor-negative human breast cancer cells. a, estrogen stimulation and catechol estrogen inhibition of the estrogen receptor-positive MCF-7 cells. Experimental conditions are the same as those described under "Experimental Procedures," except that quinalizarin was present in all dishes at a concentration of 10^{-6} M and the hormones or the vehicle present at the indicated concentration in the medium. Hormone challenges were: a, none; b, 10^{-7} M 2-hydroxyestrone; and c, 10^{-9} M estradiol. b, unresponsiveness of the estrogen receptor-negative cell lines MDA-MB-231 and MDA-MB-330 to both the catechol estrogen and the estrogen. Experimental conditions are the same as those described for the MCF-7 cells under "Experimental Procedures," except that quinalizarin was present in all dishes at a concentration of 10^{-6} M. Hormone challenges for MDA-MB-231 (dotted line) were: a, none; b, 10^{-7} M 2-hydroxyestrone; and c, 10^{-9} M estradiol. Hormone changes for MDA-MB-330 (solid line) were: a, none; b, 10^{-7} M 2-hydroxyestrone; and c, 10^{-9} M estradiol. ER, estrogen receptor.

DISCUSSION

The data presented indicate that 2-hydroxyestrone, the most ubiquitous catechol estrogen, possesses a growth-inhibitory activity on an estrogen-dependent human breast cancer cell line MCF-7. The evidence that this effect of the catechol estrogen is mediated through the estrogen-receptor is 2-fold. Firstly, the effect is antagonized by physiological levels of the primary estrogen estradiol in the range of 10^{-11} to 10^{-9} M (Fig. 3). Secondly, the growth-inhibitory effect of the catechol estrogen is not observed in the estrogen receptor-negative human breast cancer cell lines MDA-MB-231 and MDA-MB-330 (Fig. 5b) under conditions where the estrogen receptor-positive cell line MCF-7 responds to both the catechol estrogen and the primary estrogen (Fig. 5a). These results, together with the data on the in situ formation of the catechol estrogen from estradiol in human breast cancer tissues (23, 24) and others (2), suggest that the catechol estrogen may function as an endogenous antiestrogen, suberving a physiological mechanism by which the catechol estrogen normally exerts a negative regulatory control to oppose the agonist action of the primary estrogen.

Our data on the metabolism of the catechol estrogen in the tumor tissue indicate that the steroid is rapidly metabolized by O-methylation by the action of catechol O-methyltransferase in the tissue to form a metabolically stable but inactive derivative (Fig. 1). 2-Methoxyestrone is generally regarded as a physiologically inert metabolite of the catechol estrogen although it has been shown to possess a hypocholesteremic activity (25, 26); it is, however, devoid of the uterotropic activity (18), inactive as an effector of gonadotrophin and prolactin secretion (18), and has no affinity for the estrogen receptor (5). It seems therefore that the initial failure to observe the effect of the catechol estrogen on the cell growth and proliferation of the MCF-7 cells can be ascribed to its rapid metabolism by the enzyme. When the tissue catechol O-methyltransferase activity was diminished by the inclusion
of a catechol O-methyltransferase inhibitor, quinazilizin (19), the antiestrogenic activity of the catechol estrogen could be expressed. It has been reported that in human breast cancer tissues catechol O-methyltransferase was elevated 3- to 20-fold relative to the normal human mammary gland (23, 27).

In line with these data is our present result indicating that this enzyme activity is also greatly elevated in the cultured human breast cancer MCF-7 cells. It seems therefore likely that catechol O-methyltransferase plays a role in the overall expression of the "estrogenic" action of estradiol in many if not all estrogen target tissues where the primary estrogen is converted to the catechol estrogen or which are accessible to catechol estrogen produced elsewhere. The recent data by Hersey et al. (28) which indicates that the inhibition of uterine catechol O-methyltransferase by an inhibitor, U-0521, potentiates the action of catechol estrogen on the synthesis of induced protein is another case in point albeit in this instance the agonist activity of the catechol estrogen was apparently enhanced. Although catechol O-methyltransferase is known to be an ubiquitous enzyme its exact cellular and subcellular localization and control, especially in estrogen target tissues, has not been determined. Information on its localization and activity in relation to estrogen 2-hydroxylase could therefore add to our understanding of the role of the aromatic hydroxylase and the subsequent O-methylation reactions in the expression of estrogen action. Our present data indicating that the enzyme plays an important role in the expression of the catechol estrogen action in an estrogen-responsive cancer cell line MCF-7 is indicative of a possible role for this enzyme in the regulation of hormonal actions of estrogens in normal target tissues.

The actions of 2-hydroxyestrone in the presence of the catechol O-methyltransferase inhibitor on the MCF-7 cell cultures are similar to those exerted by the synthetic triphenylethylene estrogen antagonists such as tamoxifen. These substances also inhibit cellular proliferation in estrogen receptor-positive cells (21), an action which can be reversed at an early stage by estradiol but which also becomes irreversible upon prolonged exposure to these agents (21, 29). It has been proposed that these antagonists act via estrogen receptor-related nuclear events at the level of chromatin or at the level of nuclear receptor "processing" (30). The 2-hydroxyestrogens also mimic the synthetic nonsteroidal antagonists in their in vivo mode of action; they behave as mixed estrogen agonists-antagonists and like the synthetics are capable of acting as estrogens under certain circumstances and as antiestrogens under others (2-4).

The irreversible nature of the catechol estrogen inhibition of MCF-7 cell growth after 4 days of exposure to catechol estrogen (Fig. 4) may shed some light on the mechanism by which the catechol estrogen exerts its effect on the tumor cell growth and proliferation. It is evident that the reversible action of the catechol estrogens is mediated through some estrogen receptor-related event since the primary estrogen can reverse the action of the catechol estrogen when present simultaneously on day 1 (Fig. 3). In the absence of the estrogen, however, it is considered that the catechol estrogen affects irreversibly a component involved in the estrogen receptor-mediated event, rendering it unresponsive to the subsequent stimulation of the estrogen. The 2-hydroxyestrogens readily undergo oxidation to form powerful semiquinone-like electrophiles which can bind covalently to the nucleophilic sites of biological macromolecules (31, 32). It is, therefore, possible that the irreversible action of 2-hydroxyestrone on the cell growth of MCF-7 is the result of such covalent bonding to cellular macromolecules including presumably the estrogen receptor itself. The same mechanism for the irreversibility of action can be invoked in the case of the triphenylethylene estrogen antagonists. These substances are subject to oxidative metabolism (33) leading to phenolic derivatives with the potential for similar covalent binding either directly or via further transformed derivatives. In this context it is interesting that Reddel et al. (34) have reported the reversal of the inhibitory action of 4-hydroxytamoxifen on MCF-7 cell growth requires many fold greater concentrations of estradiol than in the case of tamoxifen. It is also possible that the action of the catechol estrogen may also be mediated through its binding to an antiestrogen binding site reportedly found in the cytosol of many estrogen-responsive cells (35) including the MCF-7 human breast cancer cells (36), which is distinct from the classical estrogen receptor (35, 37). Until the mechanism of catechol estrogen on antiestrogen action on MCF-7 cells is more precisely known, it is not possible to define whether the noted similarities reflect common biological pathways or whether the catechol estrogen acts as the endogenous analogue of the triphenylethylene drugs.

The present data provide evidence that 2-hydroxyestrone, a natural and qualitatively significant estrogen, can inhibit the growth of the estrogen-dependent human breast tumor cell line MCF-7. This isaccompanied by a concentration of the enzyme which although not physiological, is orders of magnitude lower than any inhibitory concentration of the other estrogens. As noted previously the exposure to catechol O-methyltransferase and biological availability of endogenous intracellularly produced catechol estrogen may differ greatly in a quantitative sense from exogenously supplied 2-hydroxyestrone. The mechanism of this inhibition is unclear but strong evidence suggests the involvement of estrogen receptor. What is clear, however, is that the action of the catechol estrogens is inhibited by the extraordinarily high activity of catechol O-methyltransferase present in these tumor cells. It is tempting to speculate that this distortion in catechol O-methyltransferase activity may have been one of the precipitating events which led to the disruption of cellular control of estrogen expression and to eventual cell transformation. The clear estrogen agonist activity of the 16α-hydroxylated metabolites of estradiol which are unaffected by catechol O-methyltransferase presence but which regulate its activity emphasize further that the direction of estradiol metabolism either in situ or in the periphery can have a profound influence on the expression of its hormonal activity.

REFERENCES


M. Huh and J. Fishman, unpublished observation.
34. Reddel, R. R., Murphy, L. I., and Sutherland, R. L. (1983) Cancer Res. 43, 4618–4624
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