Prevention of estrogen carcinogenesis in the hamster kidney by ethinylestradiol: some unique properties of a synthetic estrogen

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Introduction

Ethinylestradiol (EE*) is perhaps the most commonly prescribed synthetic estrogen, as it is employed in nearly all oral contraceptive (OC) preparations as mestranol. It is estimated that OCs have been used by 200 million women worldwide (1). EE possesses an estrogenic potency, based on standard rodent uterotrophic assay, $-5.0$–$10.0$-fold $>17\beta$-estradiol (E$_2$) and diethylstilbestrol (DES), respectively (2,3). Interestingly, except in a certain subset of young women, there is now a general consensus, based on numerous epidemiologic studies, that OCs are not associated with an increased risk for breast cancer, in either nulliparous or parous women (3–7). On the other hand, other estrogens such as, DES or E$_2$, as well as Premarin (mainly estrone sulfate and other conjugated estrogens), have been reported to increase the relative risk for this prevalent cancer if used for $>5$ years (8–10). Nevertheless, EE has been shown to increase the risk of endometrial cancer in preparations that do not contain progestins (11), as have other widely prescribed estrogens. In addition, prolonged use of EE has been primarily implicated in the relatively infrequent occurrence of hepatic neoplasms in women (12,13). The paradoxical effects of EE in human breast and liver cancer etiology, evident from numerous epidemiologic studies, have an interesting parallel in estrogen-induced neoplasms in the hamster kidney and liver.

Renal carcinomas are induced specifically in male Syrian hamsters by virtually all potent natural and synthetic estrogens after 6.0–9.0 months of treatment (14–16), with essentially a 100% tumor incidence. The major exception is EE, which yields only a 10–15% kidney tumor incidence following similar treatment periods (16). It is noteworthy, however, that the low renal tumor incidence observed following EE treatment in hamsters, required markedly higher levels of EE exposure and, thus, the presence of even greater biological potency relative to either E$_2$ or DES. Therefore, when equi-biological potencies of these estrogens are administered to separate groups of hamsters, no kidney tumors are observed after 9.0–10.0 months of EE treatment (J.J.Li and S.A.Li, unpublished data). When different estrogens were evaluated for their hepatocarcinogenicity in the hamster, only EE treatment exhibited appreciable carcinogenic activity (20–30% tumor incidence) when administered as the sole etiologic agent (17). Moreover, EE is the most potent carcinogenic estrogen in the hamster liver when administered concomitantly with either α-naphthoflavone (17–19) or ethanol (S.A.Li, unpublished data), resulting in essentially 100% hepatic tumor incidence. However, no liver tumors were observed when either E$_2$ or estrone were co-administered in these treatment regimens.

It has been established that the hamster kidney is a bona fide estrogen-responsive and -dependent tissue (14–16, 20–22). As is characteristic of estrogen target tissues, estrogen treatment induces both renal estrogen receptor (ER) and progesterone (PR) receptors in the hamster (20–22). Furthermore, it is significant that, at physiological concentrations,
estrogen administration specifically induces proximal renal tubular (PRT) cell proliferation in culture under serum-free chemically-defined conditions, and that this tubular cell growth is inhibited by anti-estrogen and androgen treatment (23,24). Therefore, it is not surprising that other agents known to oppose estrogen action, such as progestosterone, androgens and anti-estrogens, completely prevent estrogen-induced renal tumorigenesis in the hamster (25–27). The present report details for the first time the complete inhibition of estrogen tumorigenesis in the hamster (25–27). The present report details for the first time the complete inhibition of estrogen tumorigenesis in the hamster (25–27).

Materials and methods

Chemicals and reagents

Radio-inert chromatographic grade E2 was purchased from Calbiochem (Behring, CA), while all other non-labeled estrogens were obtained from either Sigma Chemical Co. (St Louis, MO) or Steraloids (Wilton, NH). All estrogens used exhibited purity of >95% as shown by high pressure liquid chromatography (HPLC) analyses using a Waters model 840 liquid chromatography equipped with a Waters 490 programmable multi-wavelength detector (2,4,6,7-[3H(N)]-17β-estradiol (70–100 Ci/mmol), [6,7-3H(N)]jestradiol (40–60 Ci/mmol), and [α-32P]CTP (800 Ci/mmol) were purchased from DuPont NEN Research Products (BOSTON, MA). Restriction enzymes T3 and T7 polymerase were purchased from Promega (Madison, WI), and Hybond nylon membranes from Amersham (Arlington Heights, IL). cDNAs were generously provided by the following investigators: hamster c-fos and c-myc, Dr Jeff Boyd, University of Pennsylvania Medical Center, Philadelphia, PA; mouse c-jun and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Dr James P.Calvet, University of Kansas Medical Center, Kansas City, KS. Primary antibodies used were anti-human c-MYC (C-33, Ab-1 mouse monoclonal IgG1), c-FOS (2G9C3, Ab-1 mouse monoclonal IgG2), and c-JUN (KM-1, Ab-1 mouse monoclonal IgG2) from Santa Cruz Biotechnology, Inc., Santa Cruz, CA. The secondary antibody was goat anti-mouse IgG horseradish peroxidase conjugate from Oncogene Science, Inc. (Cambride, MA). All other chemicals and solvents used were of the highest purity available and obtained from either Sigma Chemical Co. (St Louis, MO) or Fisher Scientific (Chicago, IL).

Animals and treatments

Adult castrated male Syrian golden hamsters (LAK:LVG), outbred strain, weighing 90–100 g were purchased from Charles River Lakeview Hamster Company (Wilmington, MA). Animals were housed in facilities certified by the American Association for the Accreditation of Laboratory Animal Care. They were acclimated for at least 1 week prior to use, maintained on a 12-h light:dark cycle and fed certified rodent chow (Ralston-Purina 5002) and tap water ad libitum. The animal studies were carried out in adherence to the guidelines established in the ‘Guide for the Care and Use of Laboratory Animals’, US Department of Health and Human Resources (NIH 1985). Hamsters in the treatment groups were implanted s.c. with 20-mg estrogen or tamoxifen (TAM)-treated, and age-matched untreated castrated male hamsters (six/group) were quickly excised, immediately frozen in liquid nitrogen and stored at − 80°C until assayed.

Probe labeling

[α-32P]CTP-labeled riboprobes were generated in vitro using T7 or T3 polymerase with linearized cDNA subclones according to the supplier’s recommendations. The labeled products were purified by Sephadex G-50 Quick-spin columns (28).

Preparation of RNA

RNA was prepared by the method of Chomczynski and Sacchi (29) with some modifications. Briefly, tissue samples were homogenized for 60 s in 5 ml of 5 M guanidinium isothiocyanate using a Polytron (Brinkmann Instruments, NY) set at maximum speed, followed by phenol-chloroform extraction. The RNA was precipitated with isopropanol and dissolved in RNase-free water. RNA concentration was determined by absorbance at 260 nm.

Northern blot analysis

In order to reduce the effect of individual variation, Northern blots were prepared using RNA pooled from three hamsters/group. Additional pooled samples were obtained for each group to confirm results obtained. Briefly, 10 µg of denatured total RNA were loaded onto each lane and fractionated in 2.2 M formaldehyde--1.5% agarose gel. All gels were stained with acridine orange and photographed to ascertain the integrity of RNA samples and to confirm that equal amounts of RNA were loaded. RNA was then transferred to Hybond nylon membranes using a capillary transfer consisting of 10% SSC (1× SSC: 150 mM NaCl, 15 mM sodium citrate, pH 7.0) and UV cross-linked with total energy of 0.3/formamide, 0.5% dextran sulphate and 50 µg/ml yeast RNA for 3 h at 65°C, then hybridized in prehybridization buffer containing 10 8 cpm/ml eRNA probe for 16–18 h at 65°C. Blots were washed for 1 h in 0.3× SSC/0.1% SDS at 65°C and twice for 1 h in 0.1× SSC/0.1% SDS at 65°C, then exposed to X-ray film with or without intensifying screens. Control for equal loading and blotting of RNA was performed by stripping blots in boiling 0.05× SSC/0.1% SDS, and rehybridizing with anti-sense riboprobe generated from cDNA clones for GAPDH. The hybridization signals were scanned and quantified with a Personal Densitometer SI (Molecular Dynamics, Sunnyvale, CA). The blots are representative of a minimum of three individual experiments, using pooled samples from a different group of three hamsters in each experiment.

Western blot analysis

For Western blot analysis of c-MYC and c-FOS, kidney cytosolic fractions pooled from at least three hamsters/group were used. Additional pooled samples were obtained for each group to confirm results obtained. Tissue samples were homogenized by Polytron using the following buffer: 10 mM Tris-HCl (pH 7.4) with 1.5 mM EDTA, 1 mM DTT, 10% glycerol, 5 µg/ml leupeptin, 5 µg/ml aprotonin, 1 µg/ml pepstatin and 100 µg/ml bacitracin. The homogenates were fractionated by subsequent spins at 1000 g for 15 min and at 100,000 g for 60 min. Cytosolic protein concentrations were determined using the BCA method with BSA as standard. Protein aliquots (100 µg) from each sample were applied to 10% or 4–20% gradient Tris-glycine SDS-PAGE (Novex, San Diego, CA) for electrophoresis under reducing and denaturing conditions (30). Thereafter, proteins were transferred to nitrocellulose membranes which were treated with primary antibodies followed by secondary antibodies conjugated with horseradish peroxidase. Immunoblots were visualized from the medullas. The method for isolating renal tubules has been described in detail elsewhere (23,24). The prepared kidney tubules were suspended in a chemically-defined serum-free medium as described in a later section. Light microscopic analysis of paraffin-embedded renal tubular preparations exhibited a consistency of >97% proximal tubules.

Culture of primary renal tubules

HR-9 basement membrane-coated flasks were prepared according to a method described previously (23). Renal tubules at a concentration of ~500/flask were plated in Waymouth’s MB 752-1 medium supplemented with insulin (5 µg/ml), transferrin (5 µg/ml), triiodothyronine (7 µg/ml), hydrocortisone (0.18 µg/ml), and epidermal growth factor (5 µg/ml) from Sigma; selenium (5 µg/ml) from Life Technologies Inc., Rockville, MD; and penicillin (100 units/ml) and streptomycin (100 µg/ml) (GIBCO). No serum was added to the culture medium. Fresh medium (1.0 ml) was added to each flask every other day. Flasks were maintained at 37°C in an humidified incubator equilibrated with 95% air/5% CO2. Morphology of the cultures was monitored daily by phase-contrast microscopy and periodically by transmission electron microscopy.

Tissue isolation for proto-oncogene expression

Whole kidneys from 5-month DES-, EE-, TAM-, DES + EE- and DES + TAM-treated, and age-matched untreated castrated male hamsters (six/group) were quickly excised, immediately frozen in liquid nitrogen and stored at −80°C until assayed.

Preparation of RNA

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and 10.0-month DES- or E2-treated hamsters, respectively, as previously revealed. Cytosol fractions from kidneys and pure renal carcinomas obtained from 4.5-ER competitive binding studies were carried out on filtered (Millipore, 25 µm) cytosol fractions. SDS-PAGE electrophoresis, followed by immunoblotting, was used to evaluate the concentration and quality of the extracts. The blots are representative of a minimum of three individual experiments, using pooled samples from a different group of three hamsters in each experiment.

Immunoprecipitation
For the immunoprecipitation of c-JUN, kidney cytosolic fractions pooled from at least three hamsters/group were used (31). Aliquots (300 µg total protein) were incubated with primary antibodies, then with Protein A/G Plus-Agarose (Santa Cruz Biotechnology, Inc., CA). The complex was collected, washed, and then broken by treatment with 2 M NaSCN. The protein A/G PLUS-Agarose complex was spun down, and the antigen precipitated with 8% TCA/0.02% Na deoxycholate, washed with ethanol/ether (1:1), and dried under vacuum. The dried antigen was resuspended in sample buffer, subjected to SDS-PAGE electrophoresis, followed by immunoblotting.

Competitive binding studies
ER competitive binding studies were carried out on filtered (Millipore, 25 µm) cytosol fractions from kidneys and pure renal carcinomas obtained from 4.5- and 10.0-month DES- or E2-treated hamsters, respectively, as previously described by us (14,20). All estrogen pellets were withdrawn 63 h prior to killing the animals to clear endogenous hormone. Cytosolic fractions were treated with 5 nM [3H]-17β-E2 or -EE in the presence or absence of various concentrations (25, 50 and 500 nM) of radio-inert natural (E2) or synthetic estrogens (DES, EE, MOX). Dextran coated-charcoal was used to remove free hormone and lower-affinity binding components. Cytosolic protein concentrations were determined using the BCA method with BSA as standard. The results represent the average of three individual determinations using different groups of three hamsters each.

Statistical analyses
The significance of differences between experimental groups was determined by Student’s t-test.

Results
Effect of EE on hamster kidney morphology
In the hamster kidney, EE induces morphologic alterations markedly different from those observed after treatment with either DES or E2 (Figure 1). After only 2 weeks of EE treatment, a distinctive hyperplasia was seen in subsets of proximal tubules residing deep within the renal cortex, which was uncommon following either DES or E2 treatment. The nuclei of these EE-induced hyperplastic cells were larger, basally located and exhibited disperse chromatin compared to unaffected normal proximal tubular cells. With continued EE treatment (>12.0 months), these renal hyperplasias displayed progressive dysplasia, but no tumors.

Prevention of estrogen carcinogenicity by concomitant EE treatment
After 8 months of hormone treatment, potent carcinogenic estrogens such as DES, E2 and MOX elicited 100% renal tumor incidence in male hamsters with a total of 13–18 tumor foci per animal (Table I). These results are similar to those we reported elsewhere (15,20,24). Remarkably, when EE was administered concomitantly with each of these potent carcinogenic estrogens, estrogen-induced renal tumorigenesis was specifically and completely abolished (Table I). Similar complete renal tumor inhibition has been shown in hamsters treated with estrogens in combination with anti-estrogens, progesterones or androgens (25–27). When the pellet dose (administered every 2.5 months) of DES or E2 was doubled that of EE, renal tumor foci were still not detected after examination of esterase stained frozen serial sections (data not shown). Additionally, combined treatment of DES and either 17β- or 17α-E2, the latter estrogen being non-carcinogenic in the hamster kidney, did not substantially alter the high frequency of renal induction or the number of tumor foci seen in animals treated with DES alone (Table I).

Effect of EE alone and in combination with E2 on renal tubular cell proliferation in vitro
To begin to assess the underlying cellular and molecular mechanisms involved in the prevention of estrogen carcinogenesis by EE, the rate of cell proliferation of cultured primary untreated hamster PRT cells grown under serum-free chemically-defined conditions was examined after treatment with E2 and EE, alone and in combination, at days 3, 10 and 21 after attachment (Figure 2). At 10 and 21 days in culture, E2 (1.0 nM) elicited a significant (P < 0.05) 1.7-fold increase in PRT cell proliferation relative to untreated control PRT cells. However, PRT cells exposed to EE at a concentration of 1.0 nM, did not respond in the same proliferative manner as that observed after either E2 (Figure 2) or DES exposure (data not shown). Combined treatment of PRT cells with increasing concentrations of EE (0.50 and 1.0 nM) plus E2 (1.0 nM) resulted in significant inhibition (P < 0.001), nearly 50%, of PRT cell proliferation relative to E2 alone at 10 and 21 days after attachment. Even when a lower dose of EE (0.25 nM) was co-administered with E2 (1.0 nM), the prolifera-

| Table I. Prevention of estrogen carcinogenicity by ethinylestradiol |
|-------------------------|------------------|------------------|
| Estrogena (%) | % Tumor induction | Combined no. of tumor nodules in both kidneys |
| DES | 100 | 15 ± 2.0 |
| 17β-Estradiol (β-E2) | 100 | 13 ± 1.0 |
| Ethinylestradiol | 10 | 2 ± 1.0 |
| Moxestrol | 100 | 18 ± 4.0 |
| DES + EE | 0 | 0 |
| β-E2 + EE | 0 | 0 |
| Moxestrol + EE | 0 | 0 |
| DES + DES | 100 | 12 ± 2.0 |
| DES + β-E2 | 100 | 12 ± 2.0 |
| DES + α-E2 | 100 | 9 ± 2.0 |

aDuration of estrogen treatment was 8 months. Each treatment group contained 8–15 animals. Data represent the mean ± SEM.

b Determined by microscopic examination of esterase activity in tumor nodules compared to surrounding normal tissue (frozen serial sections, 30 sections per kidney).
Figure 2. Effect of different estrogens, alone and in combination on primary renal proximal tubular cell outgrowth on PF-HR9 basement membrane in serum-free chemically defined medium. Hamster tubules (~500/flask) were plated in separate 25-cm² flasks, in the absence or presence of estrogens. After attachment of the tubules (5 days), the explants and monolayer were allowed to grow. Later, they were washed and treated with 0.1% EDTA on days 3, 10 and 21 (days 8, 15 and 26 after culture initiation), to desegregate them into single cells. The cells were counted in a hemocytometer. Data represent the mean ± SEM of six counts from three individual flasks. (C) No hormone treatment; (E) 17β-estradiol 1.0 nM; (EE) ethinylestradiol 1.0 nM; E₁EE, 0.25 nM, 0.50 nM and 1.0 nM.

Comparison of PRT cells was significantly reduced (P < 0.001) at 21 days.

Relative protooncogene expression after treatment with EE or TAM alone and in combination with DES

Estrogen-responsive protooncogene RNA expression was examined after 5 months of in vivo treatment with DES and EE, alone and in combination, as well as with TAM and TAM + DES (Figures 3 and 4A). After DES treatment, hamster kidney c-myc, c-fos and c-jun RNA rose 3.2-, 4.1- and 3.1-fold, respectively, relative to the levels seen in age-matched controls. Similar over-expression of these proto-oncogenes in the hamster kidney was observed after 5 months of E2 treatment (data not shown). In contrast, EE exposure alone or in combination with DES for the same treatment period resulted in consistently lower levels of RNA expression of these protooncogenes, 1.4–1.9-fold and 1.4–1.6-fold, respectively (Figures 3 and 4A). In the EE + DES-treated hamsters, c-myc, c-fos and c-jun RNA expression was reduced 43, 37 and 52%, respectively, compared to levels observed after DES treatment alone. Interestingly, TAM alone or TAM + DES treatment resulted in essentially the same low levels of protooncogene RNA expression as those seen after treatment with EE alone or in combination with DES, 0.8–1.5-fold and 0.6–1.8-fold, respectively (Figures 3 and 4A). It is noted from these data that c-fos RNA expression appears more responsive to TAM inhibition.

c-MYC, c-FOS and c-JUN protein changes after treatment with EE or TAM alone and in combination with DES

Consistent with the preceding findings of early response protooncogene expression in the hamster kidney after 5 months of DES, EE and TAM treatment alone or in combination, c-MYC, c-FOS and c-JUN protein products were similarly altered (Figures 5 and 4B). When compared to age-matched control levels, DES treatment induced 2.7-, 4.6-, and 2.9-fold increases in c-MYC, c-FOS, and c-JUN proteins, respectively, while EE treatment resulted in only a small rise, 1.3- to 1.6-fold, in these protein levels. However, after EE treatment alone, the protein levels of c-MYC, c-FOS and c-JUN were reduced to 59, 31 and 45%, respectively, of those seen after DES treatment alone (Figure 5). Interestingly, after treatment with DES + EE, the levels of these proteins were as low as those observed after EE alone. Hamsters treated with either TAM or TAM + DES also exhibited similarly low levels of these gene protein products in the kidneys compared with the elevated levels seen after DES treatment alone. The level of c-FOS protein, however, was reduced 17 to 19% compared with that found in DES-treated kidneys, reflecting the lower level of mRNA c-fos expression observed in TAM- and TAM + DES-treated animals.

Estrogen receptor competitive binding studies

In an effort to discern whether the differential binding of various estrogens (E₂, DES, EE, MOX) to renal ER contributes to our understanding of the prevention of estrogen carcinogenicity in the hamster kidney by concomitant EE treatment, competitive binding studies were carried out (Figure 6). In the presence of radioactive E₂ (Figure 6A), primary renal tumor ER exhibited comparable competitive binding for E₂, EE and DES (58 to 94%), at concentrations ranging from 5- to 100-
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Fig. 4. Densitometric quantitation of the relative expression of kidney RNA protooncogenes (A) and their respective protein products (B) in the hamster kidney after 5 months of estrogen and/or anti-estrogen treatment. The Northern and Western blots shown in Figures 3 and 5, respectively, were quantified by densitometric scanning. The relative expression represents the ratio of the densitometric values for treated versus age-matched control kidney samples.

Fig. 5. Representative Western blot analysis of the relative expression of c-MYC, c-FOS and c-JUN in the hamster kidney after 5 months of estrogen and/or anti-estrogen treatment. Pooled cytosolic protein fractions from three hamsters/group treated with DES (D), EE, DES + EE (D + EE), tamoxifen (T), tamoxifen + DES (TD), 17β-estradiol (E2) and age-matched untreated controls (C) were prepared. Protein fractions (100 µg aliquots) were examined by Western analysis (c-MYC and c-FOS) or by immunoprecipitation (c-JUN) as described in the Materials and methods section.

Fig. 6. Representative competitive binding of different concentrations of radio-inert estrogens (25, 50 and 500 nM) for estrogen receptor in the presence of 5 nM [3H]E2 (A) or [3H]EE (B) carried out on cytosolic fractions obtained from 4.5-month DES-treated hamster kidneys and estrogen-induced renal carcinomas after 10 months of DES treatment. Estrogen pellets were withdrawn 63 h prior to harvesting the tissue. The results represent the average of three separate determinations performed in groups of three hamsters each, in which [3H]E2 or -EE binding without competitor corresponded to 0% inhibition.

Discussion

There is growing evidence that estrogens may not only have dissimilar biologic activities at various organ sites, and as a consequence, differing carcinogenic activities (24). Therefore, when different estrogens are adjusted in concentration to yield equivalent biologic responses in the uterus, they may not all elicit the same biological and/or carcinogenic responses in other estrogen-responsive and -dependent target tissues. For example, despite higher EE exposure and, therefore, even greater estrogenic activity than that observed after DES or E2 treatment, hamsters exposed to EE consistently exhibited lower serum prolactin levels (16). Moreover, the in vitro proliferative response of cultured hamster PRT cells exposed to 1.0 nM EE is significantly lower when compared with PRT cells treated with DES, E2 or MOX at the same concentration (24). This unexpected effect of EE in hamster PRT estrogen-responsive cells is consistent with its low to negligible carcinogenic activity in the hamster kidney. Moreover, EE treatment for 5 months resulted in a significantly lower frequency of kidney chromosomal aberrations (chromatid breaks and exchanges, chromosome breaks and exchanges, and incidence of endoreduplicated cells) compared with that observed in animals treated for similar periods with DES, E2 , or MOX, despite even higher levels of exposure to EE (32). These data clearly illustrate

fold excess, although MOX was less effective at 5- and 10-fold excess concentrations (35 and 46%, respectively). However, at all concentrations tested, both EE and MOX showed less ability (15–45%) to displace labeled E2 from renal ER prepared from 4-month DES-treated hamsters than either E2 or DES (18–76%). On the other hand, when EE was the radiolabeled ligand (Figure 6B), at all concentrations tested, every radio-inert estrogen studied demonstrated comparable abilities to displace radioactive EE from both 4-month DES-treated kidney ER (30–91%) and primary renal tumor ER (35–85%).
significant biological differences between EE and TAM exhibit
uterine RNA over-expression of c-fos, c-jun, junB and c-myc which peak at ~6 h after treatment (33). Similarly, in human endometrial carcinomas grown in nude mice, TAM also induced c-fos RNA expression (34). Therefore, the pharmacological effects of TAM are similarly dependent upon the species and tissue endpoints under study, as well as the treatment regimen (e.g. dose, acute versus chronic exposure). Moreover, when TAM and E2 are given simultaneously, this anti-estrogen blocks the uterine response induced by the estrogen (35). Therefore, the suppression of hamster PRT cell growth observed in culture after EE treatment alone and in combination with E2, indicates that EE possesses novel anti-estrogen-like properties in the hamster kidney. It is anticipated that, in this model system, the anti-estrogen-like effects of EE are due to mechanism(s) distinct from those established for anti-estrogens such as TAM. Since, in the presence of radioactive EE, comparable competitive binding abilities to either estrogen-treated kidney or renal tumor ER were detected among the various estrogens studied, these data indicate that differential binding of EE to hamster kidney ER, relative to the other estrogens tested, does not significantly contribute to the prevention of estrogen carcinogenesis at this organ site by EE. On the other hand, the lower ability of both EE and MOX to displace the radiolabeled E2 from the ER derived from estrogen-treated kidneys is in contrast to the much greater competition by these estrogens observed in the renal tumor ER. These findings suggest the possibility that the ER present in these two renal tissues may be slightly different.

EE treatment alone and in combination with DES resulted in reduced RNA expression of c-myc, c-fos and c-jun, and of their respective protein products. This result is likely due to a lack of nascent and early renal tumorous foci development. Nevertheless, these data, taken together with the inhibitory effect on hamster PRT cell proliferation observed after in vitro EE treatment, strongly indicate that the prevention of estrogen-induced carcinogenesis by EE results from a critical effect on an early event in this multi-step neoplastic process. Moreover, compared with either age-matched control or DES-treated animals, treatment with EE and DES + EE for 5 months yielded only marginal increases in renal apoptotic cells, using the ApopTag technique (D.J.Liao, J.J.Li and S.A.Li, unpublished observations), indicating that apoptosis is not involved in mediating this unique effect of EE in the hamster kidney. A sequential epigenotoxic multi-step scheme for estrogen carcinogenesis in the hamster kidney has been proposed by us (32,33,36–38). This process involves initial cell proliferation of estrogen-sensitive cells, cell damage, reparative hyperplasia, aneuploidy, chromosome imbalance and aberrations contributing to genetic instability, as well as gene alterations and amplification. In the development of estrogen-induced neoplasia in the hamster kidney, it is proposed that a critical event is inappropriate gene expression and gene dysregulation, which confers a proliferative advantage on certain ER-positive multi-potential interstitial cells (39,40). Data presented herein support the notion that EE may critically alter ER-mediated pathway(s), thus preventing gene dysregulation, and hence, tumor formation in the hamster kidney. Studies under way in our laboratory involve the possible differential induction of ER-associated proteins in the hamster kidney by EE, as well as alterations in hamster kidney ER conformation resulting from interaction with EE, which differs from other known potent carcinogenic estrogens.

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