Effects of Oral Administration of Tamoxifen, Toremifene, Dehydroepiandrosterone, and Vorozole on Uterine Histomorphology in the Rat (44493)

KENNETH P. NEPHEW,*[,1] ELIZABETH OSBORNE,* RONALD A. LUBET,† CLINTON J. GRUBBS,‡ AND SOHAIB A. KHAN§

Medical Sciences, Indiana University School of Medicine, Bloomington, Indiana 47405–4401; †Division of Cancer Prevention, Chemoprevention Branch, National Cancer Institute, Bethesda, Maryland 20892–7322; ‡The University of Alabama at Birmingham, Chemoprevention Center, Birmingham, Alabama 35294–3361; and §Department of Cell Biology, Neurobiology, and Anatomy, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267–0521

Abstract. **Tamoxifen, toremifene, DHEA, and vorozole inhibit tumor growth in rodent mammary carcinoma models and are promising chemotherapeutic agents for use against breast cancer development. In the present study, the effect of these agents on uterine histomorphology following oral administration to mature ovary-intact rats (n = 380) was examined. Animals received diet only (control), tamoxifen (0.4 and 1 mg/kg of diet; 10 mg/kg BW by daily gavage), toremifene (3–30 mg/kg of diet), DHEA (24–2000 mg/kg of diet), or vorozole (0.08–1.25 mg/kg BW by daily gavage) for 28 days and were either sacrificed or returned to a basal diet and then sacrificed 21 days later. Treatment with toremifene (all doses) or tamoxifen (1 and 10 mg/kg) for 28 days produced a decrease ($P < 0.05$) in overall uterine size and myometrial thickness; however, uterine luminal and glandular epithelia cell height increased ($P < 0.05$) compared with control. These compartmentalized uterotrophic and antiestrogenic effects of toremifene and tamoxifen were still apparent after 21 days post-treatment. Administration of DHEA (2000 mg/kg of diet) for 28 days had dramatic uterotrophic effects, increasing ($P < 0.05$) overall uterine size and stimulating all three uterine compartments (epithelia, stroma, and myometrium). The other doses of DHEA, however, were not uterotrophic. Interestingly, after removal of DHEA from the diet, uterine weight and myometrial thickness decreased ($P < 0.05$). Vorozole (1.25 mg/kg) administration for 28 days had differential, compartmentalized uterine effects, producing an increase ($P < 0.05$) in epithelial cell height, a decrease ($P < 0.05$) in stromal size, but no change in myometrial thickness. After 21 days postadministration of vorozole, luminal epithelial cell height was increased ($P < 0.05$) compared with control. The data suggest that oral administration of tamoxifen, toremifene, DHEA, and vorozole results in differential, compartmentalized effects in the uterus that are highly dependent on treatment dose. The data may have implications for risk assessment of these agents prior to administration to healthy, cancer-free women.** [P.S.E.B.M. 2000, Vol 223]

Development of chemopreventives against breast cancer requires identification of potentially active agents and a comprehensive understanding of how dose and route of administration of the drugs affect breast as well as other target tissues. Promising agents for the prevention of breast cancer in disease-free women include tamoxifen (Nolvadex), toremifene (Fareston), dehydroepiandrosterone (DHEA), and vorozole (Rivizor, R83842). The efficacy of the antiestrogens tamoxifen and toremifene has been evaluated clinically as adjuvant therapy for breast cancer (1–5). Tamoxifen is the only compound known to prevent breast cancer incidence in healthy women (6). Toremifene has promise in this regard, but the agent has estrogenic effects in the reproductive tract of humans and rodents comparable to tamoxifen (7–9). Because it has been predicted that toremifene, like tamoxifen, will produce a similar 2–3-fold risk for endometrial carcinoma with long-term

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[1] To whom requests for reprints should be addressed at Indiana University School of Medicine, Medical Sciences, 302 Jordan Hall, Bloomington, IN 47405–4401. E-mail: knephew@indiana.edu

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therapy (10), it was of interest to examine the effect of dietary administration of chemopreventive doses of toremifene on uterine histomorphology.

DHEA, an adrenal 17-ketosteroid and a biosynthetic precursor to testosterone and 17β-estradiol (11, 12), protects against chemical carcinogenesis in several rodent organs (13) and has antitumor properties in rodent mammary gland carcinoma models similar to those seen after endocrine ablation (14–17). DHEA has estrogenic activity (18–21), but effects of this steroid on the different uterine compartments (endometrial epithelium, stroma, and myometrium) after dietary administration have not been investigated.

Vorozole is a highly potent and specific nonsteroidal inhibitor of the cytochrome P450 aromatase that mediates the conversion of testosterone and androstenedione to estradiol and estrone, respectively (22, 23). Vorozole inhibits tumor growth in rat mammary carcinoma models (23–26), suppresses estradiol concentrations in breast tumors (27–29), and has demonstrated efficacy in a subset of women who failed on tamoxifen breast cancer therapy (30). Because vorozole treatment alters sex steroid levels in rat models for breast cancer (25, 26), it was of interest to examine rat uterine histomorphology after vorozole treatment.

Oral administration of chemopreventive agents represents the human-dosing route and a desirable approach to drug delivery. In the present study, doses of tamoxifen, toremifene, DHEA, and vorozole displaying antitumor properties in rodent mammary carcinoma models were administered orally to ovary-intact female rats, and subsequent uterine cell-specific effects were examined.

Materials and Methods

Female Sprague-Dawley rats (n = 380) were obtained from Harlan Sprague-Dawley, Inc. (virus-free colony number 218; Indianapolis, IN). Rats arrived at 28 days of age and were immediately placed on Teklad (4%) diet. At 50 days of age, the groups of rats (n = 20 per group) were placed on various regimens of Teklad (4%, controls); tamoxifen (0.4 and 1 mg/kg of diet and 10 mg/kg body weight by gavage); toremifene (3, 10, and 30 mg/kg of diet); dehydroepiandrosterone (DHEA; 24, 600, and 2000 mg/kg of diet); vorozole (0.08, 0.31, and 1.25 mg/kg of diet); and 17β-estradiol (0.2, 2, and 20 μg/day s.c. injection). An additional 20 animals had their diets restricted to produce body weight gain reductions of 6%, 9%, and 12%. Tamoxifen, toremifene, DHEA, and vorozole were obtained from the Division of Cancer Prevention (National Cancer Institute, National Institutes of Health, Bethesda, MD). The doses of compounds used in this study were based on our previous work on their activity (16, 17, 24, 31). After a 28-day treatment period, 10 rats in each group were sacrificed. Uteri were removed, luminal fluid was expressed, and the tissue was blotted dry prior to weighing and processing as described below. Vaginal cytology samples were obtained by aspiration daily during the last 2 weeks of treatment. To examine residual uterine cell-specific effects of these compounds, the remaining rats (n = 10 per group) were given Teklad (4%) diet only (i.e., no treatment) and then sacrificed 21 days later or at 99 days of age.

At the time of sacrifice, the uterus of each animal was collected, trimmed, and weighed. A middle segment from one of the uterine horns was fixed immediately in 4% buffered paraformaldehyde for 24 hr. Tissues were then routinely processed by embedding in paraffin wax, cutting 5-μm thick transverse sections, mounting on light microscope slides, and staining with hematoxylin and eosin, as described previously (32). Luminal and glandular epithelium heights, uterine cross-sectional area, and myometrial thickness were measured in four to six uterine sections per animal, with five animals per treatment group, using a calibrated ocular and Image-Pro Plus software (Silver Spring, MD). Data were analyzed by the Student's t test. Mean values were determined plus the standard error of the mean (SEM). Results were termed significant if $P < 0.05$.

Results

The effects of the agents on total organ weight are illustrated in Table I. Uterine weight decreased ($P \leq 0.05$) after administration of tamoxifen (1 and 10 mg/kg) or toremifene (all doses) for 28 days compared with controls. Treatment with DHEA (2000 mg/kg of diet) or estradiol (20 μg/day) increased ($P \leq 0.05$) uterine weight compared with controls; DHEA (600 mg/kg of diet) tended to increase ($P \leq 0.07$) uterine weight. None of the doses of vorozole examined in this study had a statistically significant effect on

Table I. Mean (± SEM) Uterine Weight Measurements After Treatment of Rats with Tamoxifen, Toremifene, DHEA, Vorozole or Estradiol[a]

Treatment	Uterine weight (g)	
	28-day treatment	21-days post-treatment
Control	0.37 ± 0.02	0.42 ± 0.03
Tamoxifen 0.4 mg/kg	0.33 ± 0.02	0.39 ± 0.02
Tamoxifen 1 mg/kg	0.27 ± 0.01*	0.37 ± 0.02
Tamoxifen 10 mg/kg	0.17 ± 0.01*	0.30 ± 0.02*
Toremifene 3 mg/kg	0.29 ± 0.01*	0.45 ± 0.02
Toremifene 10 mg/kg	0.25 ± 0.02*	0.37 ± 0.03
Toremifene 30 mg/kg	0.17 ± 0.01*	0.33 ± 0.02*
DHEA 24 mg/kg	0.34 ± 0.02	0.41 ± 0.03
DHEA 600 mg/kg	0.48 ± 0.05	0.31 ± 0.03*
DHEA 2000 mg/kg	0.61 ± 0.03*	0.34 ± 0.03
Vorozole 0.08 mg/kg	0.42 ± 0.02	0.49 ± 0.03
Vorozole 0.31 mg/kg	0.39 ± 0.02	0.49 ± 0.03
Vorozole 1.25 mg/kg	0.32 ± 0.02	0.44 ± 0.03
Estradiol 0.2 μg	0.38 ± 0.02	0.36 ± 0.01*
Estradiol 2 μg	0.38 ± 0.02	0.36 ± 0.02
Estradiol 20 μg	0.53 ± 0.02*	0.34 ± 0.03*
BW gain reduction 6%	0.34 ± 0.03	0.46 ± 0.03
BW gain reduction 9%	0.33 ± 0.02	0.42 ± 0.02
BW gain reduction 12%	0.42 ± 0.03	0.46 ± 0.04

[a] Uterine weight measurements (mean ± SEM). An asterisk (*) indicates statistical difference from control ($P \leq 0.05$).

uterine weight; however, organ weight tended to decrease ($P \leq 0.07$) with administration of 1.25 mg/kg body weight vorozole. Body weight gain restriction had no effect on uterine weight.

The quantitative assessment of uterine compartments is summarized in Table II; representative uterine sections from rats after 28 days of treatment are illustrated in Figure 1. Although treatment with tamoxifen (Fig. 1E; 10 mg/kg gavage) or toremifene (Fig. 1D; 30 mg/kg of diet) for 28 days decreased ($P \leq 0.05$) total cross-sectional area, the height of the uterine luminal and glandular epithelium was increased ($P \leq 0.05$) compared with controls (Fig. 1A). Antiestrogen treatment decreased ($P \leq 0.05$) the size of the stroma and the width of the myometrium. Conversely, treatment with DHEA (Fig. 1B; 2000 mg/kg) increased ($P \leq 0.05$) overall uterine cross-sectional area and the size of all three uterine compartments: luminal and glandular epithelium, stroma, and myometrium. As expected, estradiol treatment (Fig. 1F; 20 μg/day) was uterotrophic overall, increasing ($P \leq 0.05$) total uterine cross-sectional area, epithelial cell height, and myometrial thickness; however, the size of the stroma was decreased ($P \leq 0.05$) compared with controls. Vorozole treatment (Fig. 1C; 1.25 mg/kg) decreased ($P \leq 0.05$) uterine cross-sectional area and stromal size, but epithelial cell height increased ($P \leq 0.05$).

It was also of interest to examine these agents for residual effects on the uterus. Compared with controls (Fig. 2A), prior administration of tamoxifen (Fig. 2E; 10 mg/kg) or toremifene (Fig. 2D; 30 mg/kg) resulted in decreased ($P \leq 0.05$) uterine weight (Table I), cross-sectional area (Table II), and myometrial thickness (Table II) at 21 days post-treatment. Tamoxifen, but not toremifene, had residual effects on the stroma; stromal size was decreased ($P \leq 0.05$) compared with controls. Toremifene, but not tamoxifen, had residual effects on the epithelium; luminal epithelial cell height was increased ($P \leq 0.05$) compared with controls. Prior treatment with DHEA (600 mg/kg; Fig. 2B) resulted in decreased ($P \leq 0.05$) uterine weight (Table I), cross-sectional area (Table II), and myometrial thickness compared with controls. In animals that had been treated with vorozole (1.25 mg/kg; Fig. 2C), luminal epithelial cell height was greater ($P \leq 0.05$), but the other uterine compartments were not different from controls. Prior treatment with estradiol (20 μg/day; Fig. 2F) resulted in reduced ($P \leq 0.05$) uterine weight, total cross-sectional area, stromal size, and myometrial thickness; however, the luminal epithelial cell height continued to be greater ($P \leq 0.05$) than controls.

Discussion

A detailed study of the response of the different compartments of the uterus to tamoxifen, toremifene, DHEA, and vorozole was carried out. Doses of the agents having antitumor properties in rodent models for mammary carcinoma (16, 17, 24, 31) were administered orally, representing the human-dosing route and a desirable approach to drug delivery. Furthermore, when possible, agents were incorporated into the diet.

Antiestrogens, depending upon the physiological status of the animal, have paradoxical agonist and antagonist activities in uterine compartments of rats and humans (7–9, 33–36). In the present study, both tamoxifen and toremifene had an antiestrogenic effect on uterine growth overall in

Table II. Mean (± SEM) Uterine Compartment Measurements After Treatment of Rats with Toremifene, Tamoxifen, DHEA, Vorozole or Estradiol[a]

28-Day treatment[b]	Total area[c] (×10⁶)	LE[d] cell height (μm)	GE[e] cell height (μm)	Stroma[c] (×10⁶)	Myometrium height (μm)
Control	4.3 ± 0.18	9.5 ± 0.20	8.3 ± 0.27	2.7 ± 0.27	108.3 ± 2.87
Tamoxifen	1.6 ± 0.29*	24.7 ± 0.55*	22.0 ± 1.06*	0.9 ± 0.06*	81.5 ± 1.58*
Toremifene	1.5 ± 0.25*	38.5 ± 1.18*	21.9 ± 1.26*	0.7 ± 0.04*	75.3 ± 0.54*
DHEA	6.8 ± 0.17*	49.4 ± 1.81*	27.6 ± 1.93*	3.4 ± 0.31*	244.9 ± 0.93*
Vorozole	3.6 ± 0.18*	19.2 ± 1.08*	16.5 ± 1.18*	1.7 ± 0.15*	118.5 ± 1.21
Estradiol	4.7 ± 0.14	19.2 ± 0.68*	17.7 ± 0.83*	1.9 ± 0.15*	258.9 ± 1.67*
21-Day post-treatment[f]	**Total area[c] (×10⁶)**	**LE[d] cell height (μm)**	**GE[e] cell height (μm)**	**Stroma[c] (×10⁶)**	**Myometrium height (μm)**
Control	4.9 ± 0.13	13.6 ± 0.11	12.9 ± 0.07	2.4 ± 0.06	150.0 ± 3.24
Tamoxifen	3.7 ± 0.07*	13.5 ± 0.04	12.9 ± 0.08	2.0 ± 0.06*	110.0 ± 6.57*
Toremifene	4.1 ± 0.05*	18.2 ± 0.10*	14.1 ± 0.05	2.1 ± 0.02	133.0 ± 4.97*
DHEA	4.1 ± 0.06*	14.0 ± 0.56	12.6 ± 0.42	2.1 ± 0.01	110.0 ± 3.34*
Vorozole	4.7 ± 0.07	19.8 ± 0.19*	12.5 ± 0.07	2.5 ± 0.04	140.0 ± 4.95
Estradiol	3.3 ± 0.07*	19.2 ± 0.05*	11.9 ± 0.05	1.6 ± 0.04*	118.0 ± 5.02*

[a] Uterine compartment measurements (mean ± SEM). An asterisk (*) indicates statistically different from control ($P \leq 0.05$).
[b] 28-Day treatment doses are: control, diet only; tamoxifen, 10 mg/kg BW/day, gavage; toremifene, 30 mg/kg diet; DHEA, 2000 mg/kg diet; vorozole, 1.25 mg/kg BW/day, estradiol, 20 μg/day, s.c.
[c] Total cross sectional and stromal areas are $\mu m^2 \times 10^6$.
[d] LE, luminal epithelium.
[e] GE, glandular epithelium.
[f] 21-Day post-treatment represents a 28-day treatment (control, diet only; tamoxifen, 10 mg/kg BW/day, gavage; toremifene, 30 mg/kg diet; DHEA, 600 mg/kg diet; vorozole, 1.25 mg/kg BW/day; estradiol, 20μg/day, s.c.) followed by a 21-day post-treatment of diet only.

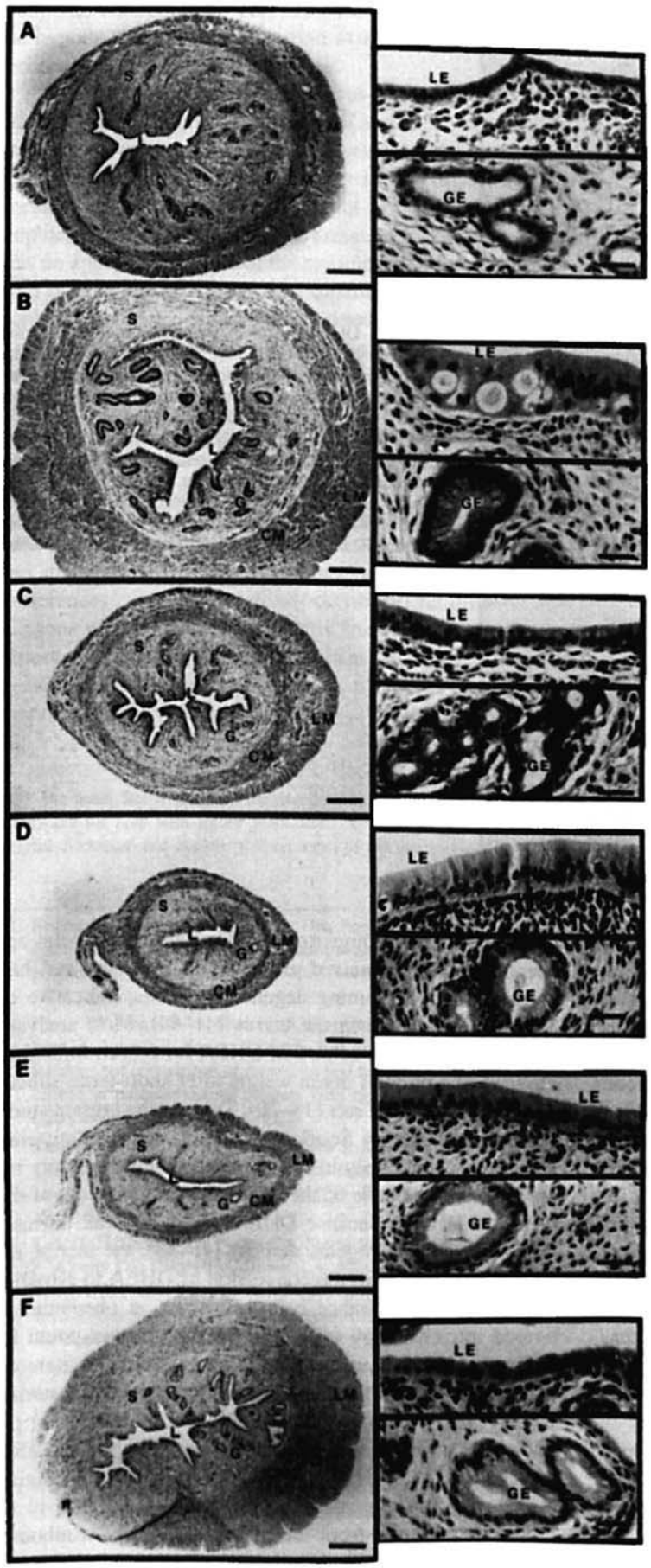

Figure 1. Uterine histomorphology after treatment with tamoxifen, toremifene, DHEA, or vorozole for 28 days. Mature, ovary-intact rats were administered agent for 28 days and then sacrificed (A) control, diet only; (B) DHEA, 2000 mg/kg diet; (C) vorozole, 1.25 mg/kg diet; (D) toremifene, 30 mg/kg diet; (E) tamoxifen, 10 mg/kg gavage; (F) estradiol, 20 µg/day s.c. LM, longitudinal muscle; CM, circular muscle; S, stroma; G, glands; L, lumen; LE, luminal epithelium; and GE, glandular epithelium are labeled. The bar equals 300 µm in cross-sections of uterine horns photographed at 50x and 30 µm in the 500x photographs. The images shown are representative sections.

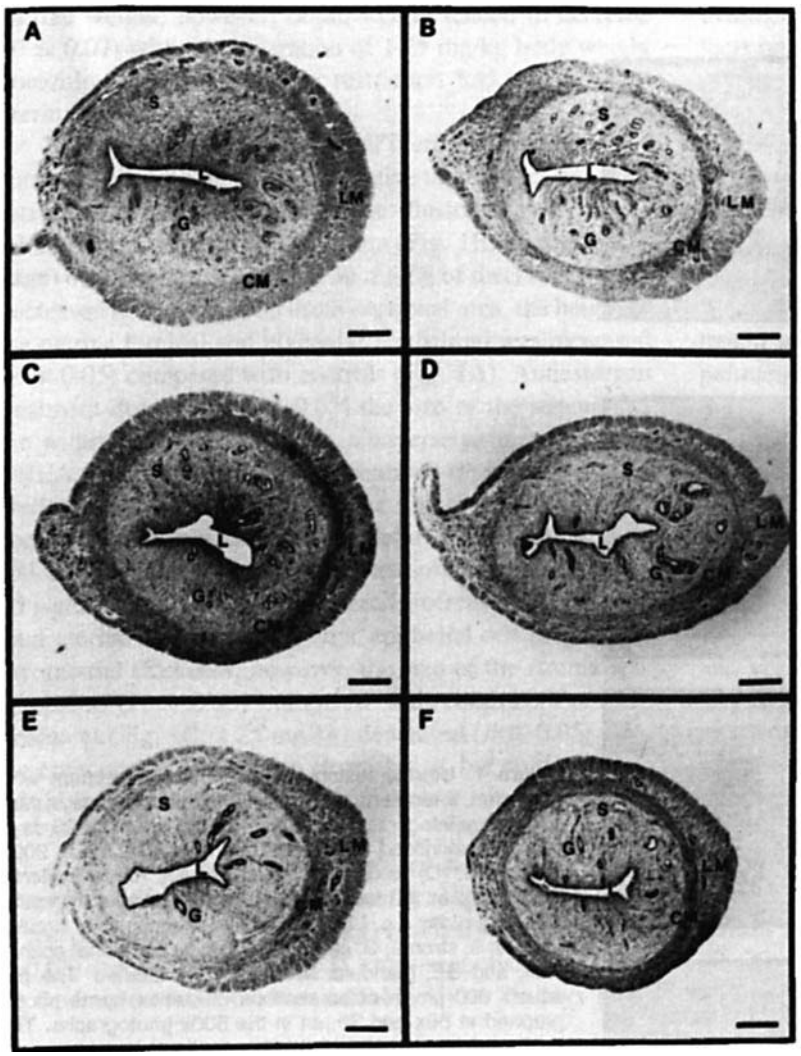

Figure 2. Uterine histology in mature, ovary-intact rats following return to basal diet. Mature, ovary-intact rats were administered agent for 28 days. After the treatment period, rats were returned to basal diet and sacrificed 21 days later. (A) diet only; (B) DHEA, 600 mg/kg; (C) vorozole, 1.25 mg/kg; (D) toremifene, 30 mg/kg; (E) tamoxifen, 10 mg/kg; (F) estradiol, 20 μg/day. LM, longitudinal muscle; CM, circular muscle; S, stroma; G, glands; and L, lumen are labeled. Cross-sections of uterine horns were photographed at 50x. The bar equals 340 μm. The images shown are representative sections.

intact rats, which agrees with previous reports on these triphenylethylenes in this animal model (36–38). Furthermore, dietary administration of toremifene decreased uterine weight in a manner similar to oral treatment of intact rats with tamoxifen (39, 40). Interestingly, both antiestrogens had uterotrophic activity in the epithelial compartment, causing marked hypertrophy of the luminal and glandular epithial cells; however, toremifene and tamoxifen had antiestrogenic activity in the stromal and myometrial compartments. Differential, compartmentalized uterine effects of toremifene and tamoxifen also became apparent after discontinuation of treatment. Toremifene had residual effects on the uterine luminal epithelia, whereas tamoxifen had residual effects on the stroma. Prolonged decreases in uterine weight after tamoxifen treatment of ovary-intact rats has been reported by others (37, 39, 40), and we report a similar phenomenon for toremifene.

The uterine effects of DHEA were highly dependent on dose. While the lower doses of DHEA, which have been shown to confer complete protection against neoplastic development in the rat mammary gland (16, 17), were devoid of uterotrophic activity, the high dose of DHEA used in this study was clearly uterotrophic, stimulating increases in size of all the uterine compartments. The uterine epithelia appeared quite disorganized after DHEA treatment and had many cavities containing degenerating cells, indicative of apoptotic cell death in the uterus (41–44). Most analyses investigating uterine effects of DHEA have been limited to observations on total organ weight after short-term, subcutaneous treatment of rats (18–20). To date, the present study and a recent one by Sourla *et al.* (21), who administered DHEA percutaneously to ovary-intact rats, are the only reports on the response of the different compartments of the uterus to DHEA. Because DHEA has only weak intrinsic estrogenic and androgenic activity (45–48), the uterine response may be due to the conversion of DHEA to estradiol or testosterone by other cells or tissues, a phenomenon termed intracrinology (49). The uterine changes could be indirect, as studies using hypophysectomized rats demonstrated that DHEA administration did not increase uterine weight (19), suggesting that DHEA acts by increasing pituitary gonadotrophin secretion and thus ovarian estradiol production (20). DHEA may also act by increasing ovarian sex steroid hormone levels (19, 50). Upon removal of DHEA or estradiol from the diet, we observed a rebound effect of the uterus, and uterine weight actually decreased

compared with controls. This rebound effect could involve the hypothalamic-pituitary-ovarian axis (50), but the exact mechanism is not clear at this time.

When uterine weight was used as the indicator of the uterine response, none of the doses of vorozole used in this study appeared to have activity. However, a differential response of the uterine compartments to the high dose of vorozole was apparent. The epithelial response was uterotrophic, the stromal response was antiuterotrophic, and there was no apparent response of the myometrium. Interestingly, after discontinuation of vorozole treatment, the luminal, but not the glandular, epithelium remained stimulated. Recent studies in ovary-intact rats showed vorozole treatment transiently decreased serum estradiol and increased serum testosterone (25, 26), and the present study suggests that the different uterine compartments may respond differently to alterations in sex steroid hormone levels by vorozole.

In summary, our results clearly demonstrate that oral administration of tamoxifen, toremifene, DHEA, and vorozole can result in differential, compartmentalized agonistic and antagonistic effects of estrogen action in the uterus. Differences in dose and delivery do not permit direct comparisons of the agents used in this study; nonetheless, the observations may be important when assessing the risks and benefits of these candidate therapies for breast cancer chemoprevention.

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