Pharmacology
Editors: K.-F. Sewing, Hannover;
E.S. Vesell, Hershey, Pa.

Reprint
Publisher: S. Karger AG, Basel
Printed in Switzerland

Original Paper

Pharmacology 1995;50:209–217

Antiestrogenic Properties of Raloxifene

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Abstract

This 21-day, open-label study evaluated the effects of raloxifene and tamoxifen on estrogen-induced changes in serum levels of anterior pituitary hormones (prolactin, luteinizing hormone, and follicle-stimulating hormone), sex steroids (testosterone, estradiol), and binding globulins [thyroid binding globulin (T_3 resin uptake), transcortin, sex steroid binding globulin]. Seventeen healthy male volunteers completed the study after being randomized to one of three treatments: raloxifene, tamoxifen, or placebo. Six subjects received raloxifene (200 mg daily) for 10 days, 6 subjects received tamoxifen [20 mg twice a day (b.i.d.)] for 10 days, and 5 subjects received placebo for 10 days. All subjects received ethinyl estradiol (20 μg b.i.d.) for 7 days starting 3 days after initiation of study drug or placebo treatment. Results of the primary analysis of this study indicate that for six of the seven analyzable parameters of estrogen action (excluding luteinizing hormone) raloxifene blunted the estrogen response; this effect was significant only for T_3 resin uptake. Tamoxifen administration significantly blunted or reversed the estrogen effect in all six of these parameters. Raloxifene, an effective antiestrogen in animal models, is also antiestrogenic in humans.

Key Words
Raloxifene
Antiestrogen
Tamoxifen

Introduction

The term antiestrogen has been applied to chemical compounds that act in various systems to antagonize estrogen action [1, 2]. In some cases, this antagonism is assumed to be via competitive binding of the antiestrogen to an estrogen receptor in target cells [3]. A particular compound may qualify as an antiestrogen because it binds competitively to an isolated estrogen receptor system, or because it demonstrates estrogen antagonistic activity in an animal model system. However, an understanding of the action of such a compound in the human subject is required before a clinical role can be defined.

Received:
August 2, 1994
Accepted:
October 19, 1994

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0031–7012/95/
0504–0209$8.00/0

Evaluation of the physiological and pharmacological effects of estrogens and antiestrogens in humans is a complicated issue. Estrogens and estrogen metabolites are normally part of the human physiological milieu. In premenopausal females, estrogen actions are affected further by complex cyclic patterns and interactions with many other endocrine systems. In postmenopausal women and in men, the situation is somewhat simplified, in that endogenous estrogen levels are low and noncycling. Administration of various forms of exogenous estrogen to postmenopausal women will suppress gonadotropin levels, stimulate binding globulins, and suppress testosterone levels [4–6]. In healthy men, estrogen administration decreases estradiol and testosterone levels; gonadotropin levels are suppressed, while binding globulin levels tend to rise [7–9].

The effects of antiestrogen administration have also been investigated in healthy women and men. Most of these studies have been done with tamoxifen, a mixed estrogen agonist-antagonist. Administration of tamoxifen to postmenopausal women does not consistently alter serum estrogen or androgen levels, but gonadotropin and prolactin levels are usually suppressed, probably due to estrogen-agonist activities at the level of the pituitary or hypothalamus. Tamoxifen administration also stimulates binding globulin in this population [4–10]. In healthy men, however, tamoxifen administration elevates levels of estrogen, testosterone, and the gonadotropins; tamoxifen may also increase levels of sex steroid binding globulin [7, 9–13].

Raloxifene is a new compound derived from a benzothiophene series of antiestrogens [14]. It competitively inhibits estrogen action in a number of in vitro and in vivo models [14–16]. In an ovariectomized rat model, raloxifene displays beneficial bone and cardiovascular effects without significant uterine effects [17]. However, the mechanism of action for this compound has not yet been established. Raloxifene displays some estrogen-like actions in addition to its estrogen-antagonistic effects [18] and can be classified as a selective estrogen receptor modulator. (Note: Raloxifene was previously studied under the name keoxifene. Two identifying numbers have also been used: LY156758 and LY139481 HCl.)

Preliminary work confirmed the literature reports that ethinyl estradiol [20 μg twice a day (b.i.d.)] administered to 5 healthy male subjects for 7 days decreased testosterone and gonadotropin levels by approximately 50% and increased sex steroid binding globulin levels by about 60% [unpubl. data]. The objective of the current study was to determine whether raloxifene would block the endocrine and biochemical changes induced by ethinyl estradiol administration in healthy male volunteers. The effects of raloxifene were compared with those of tamoxifen and placebo.

Materials and Methods

Study Volunteers

After approval of the Ethical Review Board, male volunteers between 21 and 50 years of age were recruited for the study. Detailed information about the study was provided, and written consent was obtained. Inclusion criteria specified that volunteers be within 10% of ideal body weight (as defined by Metropolitan Life Insurance tables) and in good general health as determined by a thorough medical history, physical examination, and routine laboratory tests. After this screening, 18 healthy adult male subjects were enrolled.

Study Design

This 21-day, open-label study was conducted to determine the effects of oral administration of raloxifene and tamoxifen on estrogen-induced changes in anterior pituitary hormones, sex steroids, and binding globulins. Eighteen healthy male subjects were enrolled and 17 completed the study; 1 subject in the placebo group discontinued for personal, nonmedical reasons before any measurements were taken. After baseline laboratory values were established during a 4-day base-

line period (period 1), subjects were randomly assigned to one of three treatment groups: raloxifene, tamoxifen, or placebo. During the 3 days of period 2, 6 subjects received raloxifene (200 mg daily), 6 subjects received tamoxifen (20 mg b.i.d.), and 5 subjects received placebo. During the following 7 days of period 3, all subjects received ethinyl estradiol (20 μg b.i.d.) in addition to study drug or placebo treatment. Raloxifene and placebo were administered at 7 a.m.; tamoxifen and ethinyl estradiol were administered at 7 a.m. and 7 p.m. Before, during, and after administration of these agents, plasma levels of anterior pituitary hormones, sex steroids, and binding globulins were measured.

Measurements and Other Observations

Trough blood samples were taken at 5.30 a.m. on days 1–21 to determine anterior pituitary hormones [prolactin, luteinizing hormone (LH), and follicle-stimulating hormone (FSH)], sex steroids (testosterone and estradiol), and binding globulins [thyroid binding globulin (as represented by T_3 resin uptake or T_3RU), transcortin, and sex steroid binding globulin] in all subjects.

Statistical Analysis

The primary analysis examined the combined effect of estrogen + raloxifene and estrogen + tamoxifen. The three treatment groups were analyzed with respect to changes in the anterior pituitary hormones, sex steroids, and binding globulins from period 1 (no drug) to period 3 (drug + estrogen) (fig. 1). Two secondary analyses were also performed: (1) the effects of raloxifene and tamoxifen administered alone were examined by analyzing the three treatment groups with respect to changes in markers from period 1 (no drug) to period 2 (drug alone), and (2) the effects of estrogen on raloxifene and tamoxifen were examined by analyzing the three treatment groups with respect to change in markers from period 2 (drug alone) to period 3 (drug + estrogen). The averages of the values for days 1–4 in period 1, days 5–7 in period 2, and days 11–14 in period 3 were used in these analyses. All markers were subjected to an analysis of covariance [19] on the ranked data using the value from the previous periods as the covariate.

Results

The baseline (period 1) values of the anterior pituitary hormones, sex steroids, and binding globulins were similar for the three

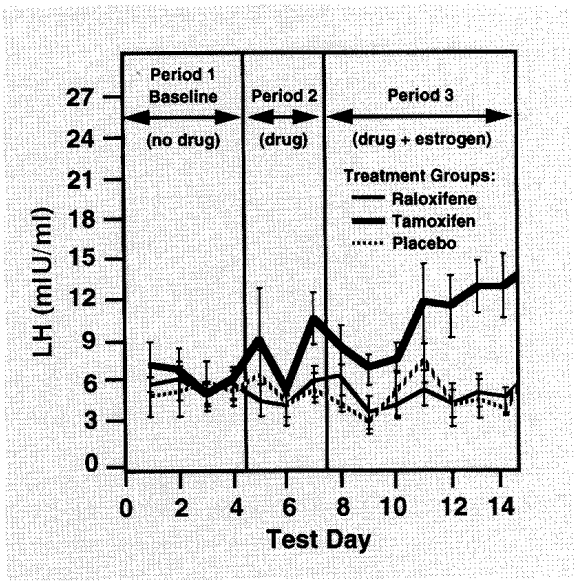

Fig. 1. Daily comparison of raloxifene, tamoxifen, and placebo for LH (mean ± standard error).

treatment groups except for FSH where the values for tamoxifen (1.74 mIU/ml) and raloxifene (2.05 mIU/ml) were significantly lower than placebo (3.47 mIU/ml).

Combined Effects of Estrogen + Raloxifene and Estrogen + Tamoxifen (Primary Analysis)

Table 1 shows the average of the differences from period 1 (no drug) to the last 4 days of period 3 (drug + estrogen) for each marker after adjusting for baseline level. The daily comparisons of raloxifene, tamoxifen, and placebo during periods 1–3 for LH, FSH, testosterone, and T_3RU are shown in figures 1–4. To evaluate the effect of estrogen alone, the within-treatment changes from period 1 to period 3 for placebo were as follows. Statistically significant increases in prolactin, transcortin, and sex steroid binding globulin were observed as well as statistically significant decreases in FSH, testosterone, estradiol, and T_3RU. No effect of estrogen on LH was

Table 1. Average differences from baseline (period 1) to mean plateau in period 3 (last 4 days)

	Placebo (n = 5)	Raloxifene (n = 6)	Tamoxifen (n = 6)	p value
Prolactin, ng/ml	3.91	2.13	−1.64[a]	0.0846
LH, mIU/ml	−0.15	−0.72	6.12[a, b]	0.0002
FSH, mIU/ml	−1.70	−0.36	1.30[a, b]	0.0027
Testosterone, ng/dl	−335.8	−226.2	−92.2[a]	0.0447
Estradiol, pg/ml	−12.68	−5.07	4.63[a, b]	0.0007
T_3RU, %	−5.27	−2.98[a]	0.30[a]	0.0105
Transcortin, ng/dl	12.09	12.86	13.80	0.6457
Sex steroid binding globulin, µg/dl	36.89	20.60	17.64[a]	0.1334

[a] $p < 0.05$, vs. placebo; [b] $p < 0.05$, vs. raloxifene.
n = Greatest number of subjects tested for any one marker.

observed; therefore, treatment comparisons for LH may not be relevant. These effects were similar when period 2 to period 3 changes were considered.

Anterior Pituitary Hormones. In the placebo group, ethinyl estradiol alone produced a significant increase of 3.91 ng/ml in prolactin levels. When raloxifene was added, this rise was blunted to 2.13 ng/ml; however, this difference was not statistically significantly different compared with placebo. Tamoxifen administration reversed the effect of ethinyl estradiol, with prolactin levels decreasing 1.64 ng/ml from period 1; this difference was statistically significant compared with placebo. As noted earlier, administration of exogenous estrogen to healthy men usually results in a suppression of plasma LH and FSH levels. In the placebo group, ethinyl estradiol produced a slight, nonsignificant, decrease of 0.15 mIU/ml in LH. A similar decrease (−0.72 mIU/ml) was seen in the raloxifene group; this effect was not statistically significantly different from the placebo group. Mean LH levels in the tamoxifen group increased 6.12 mIU/ml, a change which was significantly different from placebo and raloxifene. Ethinyl estradiol produced a significant decrease of 1.70 mIU/ml in FSH in the placebo group. A mean decrease of 0.36 mIU/ml and a mean increase of 1.30 mIU/ml were observed in the raloxifene and tamoxifen groups, respectively. The increase in the tamoxifen group was significantly different from placebo and raloxifene.

Sex Steroids. As would be expected from previous studies, ethinyl estradiol administration to these men led to a 335.8 ng/dl decrease in testosterone levels in the placebo group. Decreases in testosterone levels were 226.2 and 92.2 ng/ml in the raloxifene and tamoxifen groups, respectively; the tamoxifen results were significantly different from placebo. Consistent with literature reports, estradiol levels significantly decreased 12.68 pg/ml in the placebo group. This decrease was blunted in the raloxifene group (5.07 pg/ml); however, this change was not significantly different from placebo. Mean estradiol levels increased 4.63 pg/ml in the tamoxifen group, a statistically significant difference compared with placebo and raloxifene.

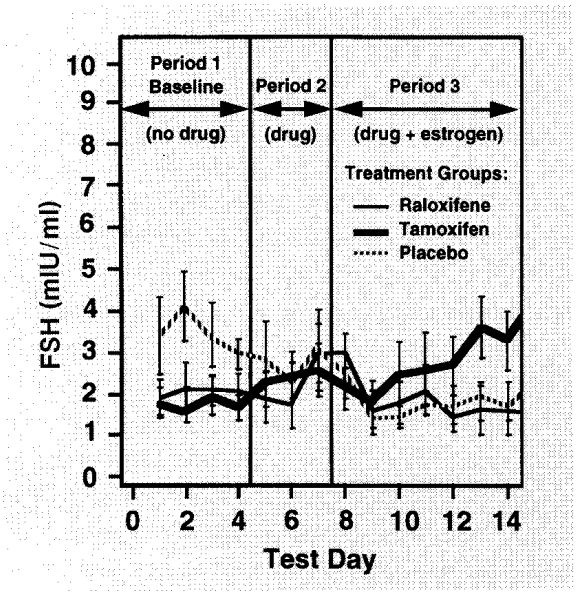

Fig. 2. Daily comparison of raloxifene, tamoxifen, and placebo for FSH (mean ± standard error).

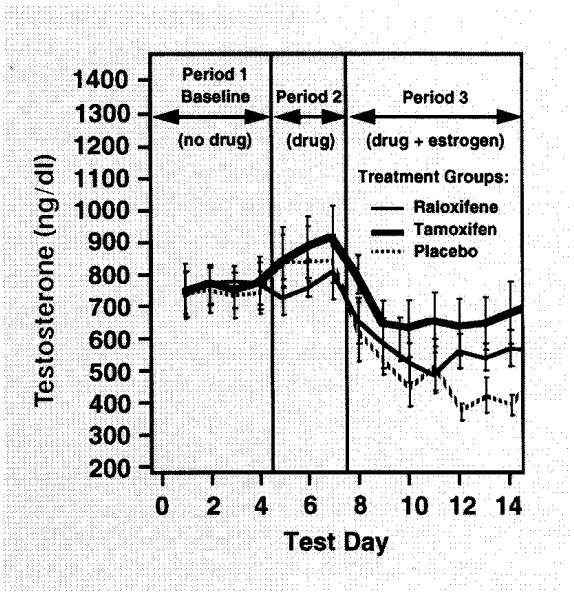

Fig. 3. Daily comparison of raloxifene, tamoxifen, and placebo for testosterone (mean ± standard error).

Binding Globulins. An increase in serum thyroid binding globulin levels will be reflected in a decrease in T_3RU. In the placebo group, ethinyl estradiol produced a significant decrease in the T_3RU (5.27%), indicating a rise in thyroid binding globulin. Decreases in T_3RU of smaller magnitude in the raloxifene (2.98%) and tamoxifen groups (0.30%) were statistically significantly different compared with the changes in the placebo group. In the placebo group, ethinyl estradiol produced a significant rise of 12.09 μg/dl in transcortin levels. The transcortin increases of 12.86 and 13.80 μg/dl in the raloxifene and tamoxifen groups, respectively, were not statistically significantly different from the increases in the placebo group. Sex steroid binding globulin significantly increased 36.89 ng/dl in the placebo group. Increases in sex steroid binding globulin in the raloxifene group of 20.60 ng/dl and the tamoxifen group of 17.64 ng/dl were blunted; the tamoxifen results were significantly different from the placebo group.

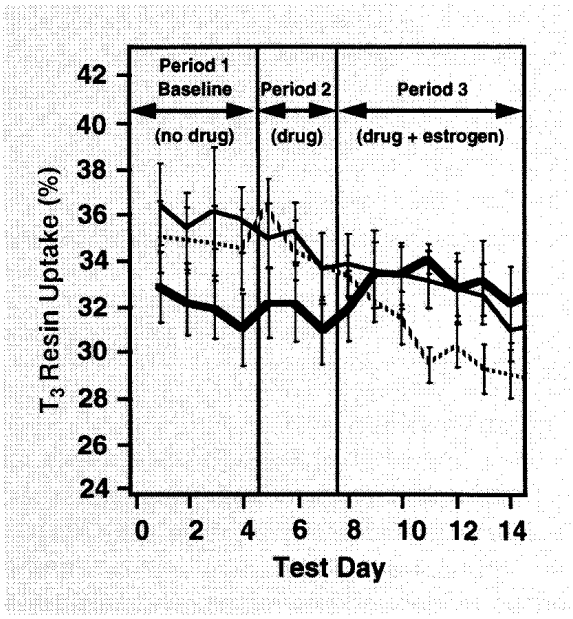

Fig. 4. Daily comparison of raloxifene, tamoxifen, and placebo for T_3RU (mean ± standard error).

Table 2. Average differences from period 1 (baseline) to period 2 (drug alone)

	Placebo (n = 5)	Raloxifene (n = 6)	Tamoxifen (n = 6)	p value
Prolactin, ng/ml	0.95	1.27	−0.35	0.7494
LH, mIU/ml	0.04	−0.76	2.13	0.2289
FSH, mIU/ml	−0.76	0.19	0.68[a]	0.0729
Testosterone, ng/dl	80.37	−4.31	135.25	0.1766
Estradiol, pg/ml	−5.89	1.96	2.00	0.3818
T_3RU, %	0.04	−1.38	−0.32	0.4826
Transcortin, ng/dl	0.73	1.70	1.86	0.3614
Sex steroid binding globulin, mg/dl	0.14	4.28	−4.12	0.6683

[a] $p < 0.05$, vs. placebo.
n = Greatest number of subjects tested for any one marker.

Table 3. Average differences from period 2 to mean plateau in period 3 (last 4 days)

	Placebo (n = 5)	Raloxifene (n = 6)	Tamoxifen (n = 6)	p value
Prolactin, ng/ml	2.96	0.85	−1.29[a, b]	0.0139
LH, mIU/ml	−0.19	0.03	3.99[a, b]	0.0034
FSH, mIU/ml	−0.94	−0.55	0.62[a, b]	0.0126
Testosterone, ng/dl	−416.1	−221.9	−227.5	0.1514
Estradiol, pg/ml	−6.79	−7.03	2.63[a, b]	0.0145
T_3RU, %	−5.30	−1.60[a]	0.63[a, b]	0.0005
Transcortin, ng/dl	11.36	11.16	11.94	0.8967
Sex steroid binding globulin, mg/dl	36.76	16.32[a]	21.77	0.0654

[a] $p < 0.05$, vs. placebo; [b] $p < 0.05$, vs. raloxifene.
n = Greatest number of subjects tested for any one marker.

Effects of Raloxifene and Tamoxifen Administered Alone (Secondary Analysis)

Table 2 shows the effects of administration of raloxifene and tamoxifen when administered alone. Even after adjusting for the period 1 imbalance, FSH was significantly higher in the tamoxifen ($p = 0.04$) group compared with placebo. No other significant effects of administration of raloxifene and tamoxifen alone were observed.

Effects of Estrogen on Raloxifene and Tamoxifen (Secondary Analysis)

Table 3 shows the differences between period 3 (drug + estrogen) and period 2 (drug alone) for each marker after adjusting for baseline level.

Anterior Pituitary Hormones. In the placebo group, ethinyl estradiol alone produced a 2.96 ng/ml increase in prolactin levels. Raloxifene blunted this increase, but the difference

was not significantly different from placebo. Tamoxifen reversed the effect of ethinyl estradiol, with prolactin levels significantly decreasing 1.29 ng/ml. In the placebo group, ethinyl estradiol produced a mean decrease of 0.19 mIU/ml in LH levels. This decrease was reversed (0.03 mIU/ml) in the raloxifene group, but the effect was not statistically significantly different from the placebo group. Mean LH levels in the tamoxifen group increased 3.99 mIU/ml from period 2, a change which was significantly different from placebo and raloxifene. Ethinyl estradiol produced a decrease of 0.94 mIU/ml in FSH levels in the placebo group. Raloxifene blunted this effect and tamoxifen reversed this effect; the change in the tamoxifen group was significantly different from the placebo group.

Sex Steroids. As would be expected from previous studies, ethinyl estradiol administration to these men led to a 416.1 ng/dl decrease in testosterone levels in the placebo group. Decreases in the raloxifene (221.9 ng/dl) and tamoxifen groups (227.5 ng/dl) were not significantly different from placebo.

Consistent with literature reports, estradiol levels decreased 6.79 pg/ml in the placebo group. This decrease was similar in the raloxifene group (7.03 pg/ml). Mean estradiol levels increased 2.63 pg/ml in the tamoxifen group, a statistically significant difference compared with placebo and raloxifene.

Binding Globulins. An increase in serum thyroid binding globulin levels will be reflected in a decrease in T_3RU. In the placebo group, ethinyl estradiol produced a 5.30% decrease in the T_3RU, indicating a rise in thyroid binding globulin. Decreases in T_3RU of smaller magnitude in the raloxifene (1.60%) and tamoxifen groups (0.63%) were statistically significantly different compared with the changes in the placebo group; the decrease for tamoxifen was also significantly different from raloxifene. In the placebo group, ethinyl estradiol produced a rise of 11.36 μg/dl in transcortin levels. The transcortin increases of 11.16 and 11.94 μg/dl in the raloxifene and tamoxifen groups, respectively, were not statistically significantly different from the increases in the placebo group. Sex steroid binding globulin increased 36.76 ng/dl in the placebo group. The increase in sex steroid binding globulin in the raloxifene group of 16.32 ng/dl was significantly different from the placebo group, but the increase in the tamoxifen group of 21.77 ng/dl was not significantly different from the placebo group.

Safety Assessments

No serious drug-related adverse events or laboratory abnormalities were observed. No clinically significant changes in heart rate or blood pressure were observed. Elevated temperature was noted in some subjects but was part of a viral syndrome and was not believed to be drug-related. No remarkable changes in routine laboratory values were observed. A mild, transient elevation in serum glutamic oxaloacetic transaminase was observed in one raloxifene-treated subject. Multiple blood sampling led to a slight fall in hematocrit in some subjects in all three treatment groups.

Discussion

In healthy men, estrogen administration suppresses estradiol, testosterone, and gonadotropin levels but stimulates globulin synthesis [7–9]. In the same population, tamoxifen administration moderately increases the levels of testosterone, estradiol, and the gonadotropins [7, 9–12]. When both estrogen and an antiestrogen are administered in the same subject, however, some 'markers' of estrogenicity might be antagonized by the antiestrogen. A partial agonist such as tamoxifen displays some estrogen-like effects which may be

synergistic to those of estrogen under certain conditions [10]. Under the conditions of this study, however, tamoxifen performed consistently as an antagonist of estrogen action.

Results of the primary analysis of this study indicate that for six of the seven analyzable parameters of estrogen action (excluding LH) raloxifene blunted the estrogen response; this effect was significant only for T_3RU. Tamoxifen administration significantly blunted or reversed the estrogen effect in all six of these parameters. There are many reasons why raloxifene may have performed differently from tamoxifen in this study, but most explanations involve either (1) assumed inherent differences between the compounds themselves, or (2) those related to the design of the study.

Some of the preclinical data on raloxifene indicates that qualitative differences from tamoxifen exist. Raloxifene and tamoxifen differ in their binding affinity for various receptors, in their ratio of agonist-antagonist action in different systems, and in other intrinsic properties [20, 21]. Raloxifene did not prevent the partial uterotropic action of tamoxifen administration [14]. Also, tamoxifen, but not raloxifene, enhanced choleragen action [15]. These differential actions could be due to differences in modes of action of the compounds themselves, or may result from differential metabolic changes.

Various aspects of study design also may be involved in explaining the observed results. Raloxifene is not well absorbed in the starch formulation, and the appropriate dosage (to see effects comparable to those of tamoxifen) may not have been administered. Neither the timing of administration nor the appropriate sampling timings were optimized, and these may have played a role in the observations of this study.

Results from the secondary analyses of this study confirm the hypothesis that raloxifene and tamoxifen each interact differently with estrogen. Comparisons between period 2 (drug alone) and period 1 (no drug, table 2) show that raloxifene and tamoxifen had similar effects on several markers and that no significant effects were observed when raloxifene and tamoxifen were administered by themselves. However, when the interaction of estrogen with raloxifene and tamoxifen (table 3) was analyzed (period 3 vs. period 2), raloxifene and tamoxifen appear to interact differently with estrogen. Tamoxifen significantly blunted the effects of estrogen in five markers: prolactin, LH, FSH, estradiol, and T_3RU, whereas raloxifene significantly blunted the effects of estrogen in sex steroid binding globulin and T_3RU. Interpretation of these results is hampered because the study design did not permit longer treatment. Stabilization of these markers following administration with either raloxifene or tamoxifen may take longer than the 3 days in period 2.

This study also provides an example of the evolution of our concepts of 'estrogens' and 'antiestrogens'. The current literature includes many examples suggesting that the traditional placement of these compounds along a spectrum of agonist-antagonist action may be misleading [2]. It may be appropriate to classify compounds such as raloxifene as selective estrogen receptor modulators. Each compound reacts with a unique set of receptors in a variety of tissues in many species. Some interactions are through 'classical' estrogen receptors, while others involve receptors to which estrogen does not bind [22, 23]. Effects on receptor dimerization, genomic control elements, and many other factors may play roles in the net action of a compound in a biological system. Genetic and metabolic differences may also be important.

The unique design of this study clearly demonstrated some of the antiestrogenic aspects of raloxifene pharmacology in man.

Properties, both desirable and undesirable, are currently being defined for appropriate application of such compounds to human disease states. Searching for a compound which will provide precisely the desired combination of clinical effects remains a formidable challenge for medicinal chemistry and pharmacology.

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