Tamoxifen and ICI 182,780 Interactions with Thyroid Hormone in the Ovariectomized-Thyroidectomized Rat

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ABSTRACT
Studies of estradiol and tamoxifen actions to modulate the actions of thyroid hormone (triiodothyronine, T3) in the rat have shown that a subset of estrogen responses require T3 for expression. Also, tamoxifen acts as a partial agonist in estrogen responses that are T3 independent, but acts as a full estrogen agonist in T3-dependent responses. This study examined whether the differing behavior of tamoxifen (a triphenylethylene antiestrogen) in T3-independent and T3-dependent estrogen responses would be shared with ICI 182,780, a steroidal antiestrogen. An ovariectomized-thyroidectomized rat model was used. Drug vehicle, tamoxifen alone (0.4 mg/kg), ICI 182,780 alone (2 mg/kg) or tamoxifen plus ICI 182,780 were given for 3 weeks to ovariectomized-thyroidectomized rats with or without T3 replacement (10 μg/kg). T3-independent estrogen responses measured were the induction of uterine growth and induction of pituitary growth hormone (GH) in the absence of T3. T3-dependent estrogen responses measured were antagonism of T3-evoked increases in pituitary GH, body weight, tibia length and hepatic malic enzyme, and increases in serum triglycerides. Tamoxifen acted as a partial agonist in T3-independent estrogen responses, whereas ICI 182,780 acted as a potent pure antagonist in such responses; it lacked agonist efficacy and totally blocked tamoxifen effects. In T3-dependent estrogen responses, tamoxifen acted as a full estrogen agonist. ICI 182,780 acted as a weak agonist in some T3-dependent responses and lacked agonist efficacy in others. Moreover, ICI 182,780 had poor efficacy in blocking tamoxifen actions in T3-dependent responses. The results indicate that ICI 182,780, like tamoxifen, displays a duality in its pharmacological behavior which pivots on the T3 dependence of the estrogen response.

Estrogens have important metabolic effects in addition to their actions to induce the growth and maturation of female reproductive organs. In women, estrogens decrease cardiovascular risk by complex effects on lipid metabolism (Nabulsi et al., 1993), and are essential for the maintenance of bone mass (Lindsay et al., 1980). Estrogens also alter somatic growth and energy, lipid and bone metabolism in rats (Wade and Gray, 1979; Wade and Schneider, 1992; Kalu, 1991; Turner et al., 1994). These metabolic actions of estrogens provide much of the rationale for their widespread use in postmenopausal women. Nonetheless, the physiological and molecular pathways responsible for these actions remain obscure.

Tamoxifen is a triphenylethylene antiestrogen with partial agonist activity in classic estrogen responses (e.g., induction of rat uterine growth) via direct binding to the ER. Tamoxifen is widely used in breast cancer therapy to antagonize estrogen-dependent tumor growth (Jordan and Murphy, 1990). Surprisingly, such therapy is associated with decreases in cardiovascular risk (Love et al., 1991) and bone loss (Love et al., 1987). Thus, tamoxifen inhibits breast cancer growth by blocking the actions of endogenous estradiol while paradoxically lessening cardiovascular disease and bone loss by apparent supplemental estrogen agonist actions. Similarly, in rats tamoxifen fully mimics estradiol effects on growth, bone mass and energy balance, but inhibits estradiol effects on the uterus and other targets (Jordan et al., 1987; Wade and Heller, 1993). This duality in tamoxifen’s behavior is difficult to understand. The nature of ER mechanisms argue against a role for “spare receptors” as a means of tamoxifen’s dual agonist-antagonist behavior (see DiPippo et al., 1995 for discussion). A second type of ER (beta) has recently been identified (Kuiper et al., 1996) and might conceivably provide a mechanism for tamoxifen’s full agonist behavior. However, tamoxifen acted as an antagonist rather than full agonist on estrogen-dependent gene expression driven by ER-beta (Kuiper et al., 1996).

Our laboratory has recently reported evidence which indicates that certain of the metabolic actions of estradiol and

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ABBREVIATIONS: T3, 3,3’,5-triiodo-L-thyronine; T4, thyroxine; TR, thyroid hormone receptor; ER, estrogen receptor; GH, growth hormone; IGF-1, insulin-like growth factor 1.
tamoxifen may arise from interference with the actions of thyroid hormone (T3) (DiPippo et al., 1995; DiPippo and Powers, 1991). With use of ovariectomized-thyroidectomized rats, it was found that estradiol and tamoxifen inhibited T3 effects on pituitary GH, somatic growth, bone and hepatic malic enzyme (an index of T3 actions on lipid metabolism), and lacked inhibitory effects on these measures in the absence of T3 (T3-dependent responses). Estradiol and tamoxifen effects to increase serum triglycerides were also completely T3 dependent. The above-mentioned responses were target- or response-selective rather than generalized: estradiol and tamoxifen did not block T3 suppression of pituitary thyrotropin secretion or T3 actions to increase pituitary prolactin. Conversely, estradiol and tamoxifen effects on the uterus, luteinizing hormone levels and pituitary prolactin and kallikrein occurred with or without T3 (T3 independent). Moreover, tamoxifen acted as a full estrogen agonist in T3-dependent responses but acted as an antiestrogen in effects that were T3 independent. Zhou-Li et al. (1992) also reported that antiestrogens can antagonize T3: 4-hydroxytamoxifen inhibited growth stimulatory effects of T3 in multiple cell lines. In addition, antiestrogens did not interfere with T3 binding to TR (Zhou-Li et al., 1992), which suggests complex mechanisms possibly related to ER-TR interactions at the transcriptional level (cross-talk). The previously noted selectivity of estradiol and tamoxifen modulation of T3 actions in animals further argues for mechanisms involving ER-TR interactions at the transcriptional level (cross-talk). The previously noted selectivity of estradiol and tamoxifen modulation of T3 actions in animals further argues for mechanisms involving ER-TR interactions at the transcriptional level (cross-talk). The previously noted selectivity of estradiol and tamoxifen modulation of T3 actions in animals further argues for mechanisms involving ER-TR interactions at the transcriptional level (cross-talk).

Steroidal antiestrogens (ICI 164,384 and ICI 182,780) have recently been developed which lack estrogen agonist activity and act as pure antagonists in classic estrogen target tissues such as the uterus (Wakeling et al., 1991; Wakeling, 1995). These drugs represent an important advance in antiestrogen pharmacology and are being evaluated for improved efficacy in breast cancer therapy. It was of interest to contrast the actions of tamoxifen (which displays partial agonist activity) with those of steroidal antiestrogens in T3-dependent and T3-independent estrogen responses (DiPippo et al., 1995).

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Materials and Methods

Animals. All procedures were approved by the institutional Animal Care and Use Committee following guidelines approved by the National Institutes of Health. Three week *in vivo* treatment protocols were used (3% of rat lifespan) to identify effects of chronic hormonal interactions potentially involving ER-TR cross-talk; efforts were made to minimize the possibility of effects arising *via* alterations in drug or hormone pharmacokinetics (see below). Female CD rats (175±200 g, Charles River, Wilmington, DE) were ovariectomized and thyroidectomized as described previously (DiPippo et al., 1995), and treatments were begun 2 weeks after the final surgery. Drinking water contained 1% calcium gluconate to maintain calcium balance and 0.025% propylthiouracil to block T3 production by any residual thyroid tissue. Propylthiouracil also inhibits deiodinases involved in T3 catabolism to diido metabolites (Kohrle et al., 1991): use of propylthiouracil reduces the possibility that drug effects may reflect alterations in such reactions. Trans-tamoxifen (free base) (0.4 mg/kg; Sigma Chemical CO., St. Louis, MO) and ICI 182,780 (2 mg/kg; Zeneca Pharmaceuticals, Macclesfield, UK) were administered sc every 24 h in sesame oil containing 10% benzyl alcohol and 20% ethanol; control animals received vehicle. Dose-response studies in ovariectomized and ovariectomized-thyroidectomized rats have shown that 0.2 mg/kg tamoxifen produces maximal estrogen agonist effects, as well as maximal antagonist effects on moderate doses of estradiol benzoate or T3 (Powers et al., 1989; DiPippo et al., 1995). Similarly, dose-response studies in the rat have shown that 0.3 mg/kg ICI 182,780 (s.c.) produces maximal inhibition of moderate estradiol doses, and a 5:1 ratio of ICI 182,780 to tamoxifen fully blocks tamoxifen’s agonist effects on the uterus (Wakeling et al., 1991; Wakeling and Bowler, 1992). A physiological replacement dose of T3 (sodium salt) (10 µg/kg; Sigma) was administered i.p. every 24 h in 0.9% NaCl containing 5 mM NaOH; control animals received vehicle. Use of T3 rather than T4 minimizes the possibility that drug-evoked changes in transthyretin and T4-binding globulin (serum-binding proteins) could alter hormone pharmacokinetics and actions because T3 has much weaker affinity for these binding proteins (Robbins, 1991). Use of T3 negates the possibility of drug effects arising from T4 deiodination to T3. Drug and T3 treatments were given for 3 weeks with five rats per group; rats were weighed daily. Rats were then killed with 100 mg/kg sodium pentobarbital (i.p.). Blood samples were obtained for serum GH and triglyceride determinations within 3 to 5 min after pentobarbital injection, and tissues were collected as described previously (DiPippo et al., 1995).

Experimental measures. Dissected uteri were stripped of fat, drained of luminal fluid and dried for 48 h at room temperature before weighing. The right tibias were stripped of all muscle and connective tissue, and stored in 0.9% NaCl at 5°C until measurement of tibia length with calipers. Pituitary and serum GH levels were measured by radioimmunoassay as described previously (DiPippo et al., 1995) with reagents provided by the National Hormone and Pituitary Program. Rat GH RP-2 was used as the standard. Hepatic malic enzyme and serum triglycerides were measured by enzymatic assay as described previously (DiPippo et al., 1995).

Statistics. Data were analyzed by one-analysis of variance followed by Duncan’s New Multiple Range Test. Where appropriate, data were log transformed to equalize variances.

Results

In the following sections, tamoxifen and ICI 182,780 are, at times, pharmacologically characterized as T3 partial agonists or T3 antagonists. It should be noted that this characterization is based on pharmacological responses involving complex, poorly understood biochemical events rather than a mechanism of direct antiestrogen-T3 competition for TR binding (see the introduction). We believe such pharmacological characterization is useful for modeling and analysis of responses arising from ER and TR interactions in complex physiological networks of responsive genes: the mechanistic distinctions should be borne in mind (also see fig. 5).

Tamoxifen and ICI 182,780 effects on uterine dry weight. Induction of uterine wet or dry weight is a classic estrogen response and has been the most widely used bioassay system for the characterization of antiestrogens. As shown in figure 1, tamoxifen almost doubled uterine dry weight in either the presence or absence of T3; this represents about 30% of the maximal induction elicited by estradiol in the ovariectomized-thyroidectomized rat (DiPippo et al., 1995). ICI 182,780 lacked estrogen agonist activity in this response and completely blocked tamoxifen induction of uter-
ine weight. T3 alone was without effect on uterine weight, and it did not alter tamoxifen or ICI 182,780 actions. These data indicate that tamoxifen and ICI 182,780 were equally efficacious in their interactions with the ER in either the presence or absence of T3; the differential behavior of tamoxifen and ICI 182,780 in T3-dependent responses (see below) is unlikely to reflect alterations in drug pharmacokinetics.

**Tamoxifen and ICI 182,780 effects on GH, body weight and tibia length.** In the absence of T3, tamoxifen induced pituitary GH levels by 10-fold; this represents about 20% of the maximal response which can be evoked by estradiol, and about 7% of the maximal response evoked by T3 (DiPippo et al., 1995). ICI 182,780 lacked effect on GH in the absence of T3 and completely blocked tamoxifen induction of GH (fig. 2). T3 induced pituitary GH by 135-fold, and tamoxifen markedly inhibited GH induction by T3 (−53%) (fig. 2). Surprisingly, ICI 182,780 also inhibited GH induction by T3 (−21%), but more weakly than tamoxifen. Moreover, ICI 182,780 failed to block tamoxifen antagonism of T3 induction of GH despite its action to block tamoxifen effects on the uterus in the same rats. It should be noted that testosterone propionate does not induce pituitary GH in thyroidectomized rats although it increases GH in thyroid-intact rats (DiPippo and Powers, 1991), apparently via hypothalamic effects (Jansson et al., 1985). This suggests that hypothalamic effects are unlikely to explain tamoxifen effects on GH. Indeed, ER is expressed in 80 to 90% of rat somatotrophs (Keefer et al., 1976; Shirasu et al., 1990), and T3, E2 and tamoxifen have been shown to directly alter GH production in pituitary cell culture (see “Discussion”).

In the absence of T3, neither tamoxifen nor ICI 182,780 significantly altered serum GH levels (fig. 2), although tamoxifen alone produced a trend toward an increase. T3 doubled serum GH levels, and neither tamoxifen nor ICI 182,780 alone altered this increase; rats treated with both tamoxifen and ICI 182,780 exhibited low serum GH levels. The poor correlation between pituitary GH content and serum GH levels is not surprising because rats can maintain normal serum GH levels as long as pituitary GH content remains at or above 10 to 15% of normal values (Peake et al., 1973; Coiro et al., 1979; Coulombe et al., 1978). Nonetheless, the data suggest that tamoxifen effects on somatic growth are unlikely to reflect altered GH secretion (see below).

In the absence of T3, rats exhibited an 18-g weight gain and neither tamoxifen nor ICI 182,780 significantly affected this gain over the course of the experiment (fig. 3). T3 produced a 76-g weight gain and tamoxifen completely blocked T3 actions to increase weight. ICI 182,780 did not alter T3 effects to increase body weight but partially blocked tamoxifen actions to inhibit weight gain (fig. 3).

In the absence of T3, tamoxifen or ICI 182,780 had no effect on tibia length, an index of longitudinal bone growth. T3 produced a 2.6-mm increase in tibia length and tamoxifen inhibited this growth by 70% (fig. 3). ICI 182,780 did not inhibit T3-induced tibia growth and also did not block tamoxif...
ifen antagonism of T3-induced tibia growth. As noted above, tamoxifen actions to alter weight gain and tibia length are unlikely to reflect the alterations in pituitary GH levels, because in this and previous studies (DiPippo et al., 1995) we have shown that neither tamoxifen nor estradiol alter serum GH in T3-treated rats despite their marked effects on pituitary GH content. On the other hand, there is considerable evidence which indicates that T3 is required for GH to fully manifest its somatotropic effects, and that estrogens can interfere with the somatotropic effects of GH (see DiPippo et al., 1995 for discussion). T3 and estrogens can also directly alter IGF-1 expression independent from GH (Dickson and Lippman, 1987; Murphy et al., 1987; Samuels et al., 1988). IGF-1 mediates much of the somatotropic effects of GH, and tamoxifen modulation of IGF-1 actions or its regulation by T3 and GH remains to be fully explored (see Pollak et al., 1992).

**Tamoxifen and ICI 182,780 effects on malic enzyme and serum triglycerides.** In the absence of T3, tamoxifen and ICI 182,780 lacked effect on hepatic malic enzyme (which supplies reducing equivalents that can be used for lipogenesis). T3 almost tripled malic enzyme (fig. 4), and tamoxifen inhibited this induction by 47% (T3 evoked a 7.5-U increase in the absence of tamoxifen compared with a 4.0-U increase in the presence of tamoxifen). ICI 182,780 reduced T3 induction of malic enzyme by 25%, but this decrease did not achieve statistical significance: ICI 182,780 did not block tamoxifen actions on T3 induction of malic enzyme.

In the absence of T3, neither tamoxifen nor ICI 182,780 affected serum triglyceride levels. T3 alone produced a 44% decrease in triglyceride levels. In the presence of T3, tamoxifen evoked a 4-fold rise in triglycerides (fig. 4); ICI 182,780 almost doubled triglycerides, and appeared to partially inhibit tamoxifen effects. It should be noted that T3 exerts complex effects on lipid metabolism, and increases both lipogenesis, lipid mobilization and lipid catabolism (Ingbar, 1985; Oppenheimer et al., 1991), with T3 actions to increase...
lipid turnover and catabolism predominating. Tamoxifen may more greatly inhibit T3 effects on lipid catabolism, and yield a T3-dependent increase in triglycerides (see DiPippo et al., 1995).

Discussion

The present study confirms our previous findings regarding tamoxifen modulation of T3 actions. Thus, tamoxifen behaves as a T3 antagonist in T3 actions to induce GH and increase body weight and tibia length. Tamoxifen also inhibited T3 induction of hepatic malic enzyme, and tamoxifen actions to increase triglycerides were entirely T3 dependent. In the absence of T3, tamoxifen lacked effect on all T3-dependent estrogen responses except for pituitary GH induction, in which the tamoxifen-ER complex appears to be completely imitated by ICI 182,780. Tamoxifen may more greatly inhibit T3 effects on lipid metabolism, and tamoxifen effects to decrease fat depots and longitudinal growth. Thus, tamoxifen would be predicted to fully mimic estradiol actions of pure antiestrogens with respect to ER binding to DNA sites. In terms of its pharmacological character, tamoxifen generally behaves as a partial agonist in estrogen responses which occur in the absence of T3 (uterine growth, pituitary GH induction), whereas tamoxifen fully mimics estrogen effects which are T3 dependent (decreases in pituitary GH levels, weight gain, longitudinal growth and malic enzyme, and increases in triglycerides) (Jordan et al., 1987; Wade and Heller, 1993; DiPippo et al., 1995; DiPippo and Powers, 1991).

The behavior of ICI 182,780 was distinct from tamoxifen. In agreement with previous results ( Wakeling et al., 1991; Wakeling and Bowler, 1992), ICI 182,780 behaved as a pure estrogen antagonist on uterine growth, lacking agonist efficacy and completely blocking tamoxifen effects in either the presence or absence of T3. Similarly, in the absence of T3, ICI 182,780 lacked agonist efficacy on GH induction and completely blocked GH induction by tamoxifen. In the presence of T3, however, ICI 182,780 partially mimicked tamoxifen effects on pituitary GH and serum triglycerides. Nonetheless, ICI 182,780 did not match the efficacy of tamoxifen in such actions and did not mimic tamoxifen inhibition of T3 effects on weight gain or tibia growth. Furthermore, ICI 182,780 was poorly effective in blocking tamoxifen actions that were T3 dependent. Overall, ICI 182,780 behaved as a potent pure antagonist in estrogen responses occurring in the absence of T3, but behaved as a weak partial agonist or was inactive in estrogen responses that were T3 dependent. Thus, ICI 182,780 shares with tamoxifen a duality in its pharmacological character which pivots on the T3 dependence of the responses.

A surprising aspect of this study was the general ineffectiveness of ICI 182,780 in blocking T3-dependent tamoxifen effects despite its ability to fully block tamoxifen effects which are not T3 dependent. For example, ICI 182,780 blocked tamoxifen induction of GH but did not block tamoxifen antagonism of T3 induction of GH. T3, estradiol and tamoxifen appear to act directly on pituitary somatotrophs to alter GH production (Komolov et al., 1980; Webb et al., 1983; Simard et al., 1986; Malaab et al., 1992). Thus, the disparate effects seem unlikely to reflect tissue-specific uptake or metabolism of ICI 182,780. Others have also reported data consistent with the phenomenon. Wakeling and Bowler (1992) found that ICI 182,780 markedly decreased uterine weight in intact female rats but failed to increase body weight, whereas ovariectomy both decreased uterine weight and increased body weight. Wade et al. (1993) found that ICI 182,780 completely blocked uterine growth induced by estradiol or tamoxifen in ovariectomized rats. In the same animals, however, ICI 182,780 only weakly blocked estradiol and tamoxifen effects to decrease fat depots and longitudinal growth. Gallagher et al. (1993) reported that ICI 182,780 blocked estradiol effects to increase uterine weight and cancellous bone volume, but lacked effect on estradiol actions to decrease body weight and bone growth in the same rats. Indeed, ICI 182,780 blocked estradiol effects on cancellous bone volume in the rat tibia while having no effect on estradiol effects to inhibit tibial longitudinal or periosteal growth (Gallagher et al., 1993). This again suggests that tissue-specific uptake or metabolism is unlikely to explain the disparate behavior of ICI 182,780.

We have proposed that T3-dependent estrogen responses may reflect ER and TR cross-talk at DNA sequences mediating receptor binding and transcriptional regulation (DiPippo et al., 1995; DiPippo and Powers, 1991). Tamoxifen may transform the ER to a form that evokes ER-TR interactions at genes with combinations of ER-TR binding elements. At such targets ER may interfere with TR actions while evoking little transactivation itself, and thus primarily act to modulate T3 action (see fig. 5). Both estradiol and tamoxifen and related triphenylethylenes can transform the ER to forms with enhanced affinity for DNA targets in vivo (Sutherland et al., 1977; Katzenellenbogen et al., 1979; Clark et al., 1973). Thus, tamoxifen would be predicted to fully mimic estradiol actions in responses arising from ER-TR cross-talk. On the other hand, antiestrogens that fail to evoke strong DNA binding would be expected to lack agonist efficacy in estrogen responses arising from ER-TR cross-talk. Interpretation of ICI 182,780 effects is complicated by controversy about the actions of pure antiestrogens with respect to ER binding to DNA (see Metzger et al., 1995). Nonetheless, there is considerable evidence indicating that ICI 182,780 decreases cellular ER levels and does not trigger ER transformation and nuclear retention analogous to that caused by estradiol or tamoxifen ( Gibson et al., 1991; Fawell et al., 1990; Reese and Katzenellenbogen, 1991; Arbuckle et al., 1992; Dauvois et al., 1992; Reese and Katzenellenbogen, 1992). Thus, the poor efficacy of ICI 182,780 in T3-dependent estrogen responses seems consistent with ER modulation of TR via cross-talk.

We expected ICI 182,780 to potently block T3-dependent effects of tamoxifen. This was not observed and might reflect incomplete blockade of the ER by ICI 182,780. The 5:1 ratio of ICI 182,780 to tamoxifen may allow incremental ER transformation by tamoxifen over several days. ICI 182,780 is ineffective in preventing ER binding to DNA once the ER becomes transformed (Fawell et al., 1990; Reese and Katzenellenbogen, 1991; Dauvois et al., 1992), and thus would be unable to fully block tamoxifen-evoked ER-TR cross-talk in such conditions. Conversely, although ICI 182,780 cannot block binding of transformed ER to DNA in vitro, it blocks the binding of the transformed ER to coactivators needed for transactivation (Halachmi et al., 1994; Cavailles et al., 1994). This would enable ICI 182,780 to fully block T3-independent tamoxifen responses (which require ER-evoked transactiva-
adverse feminizing or thromboembolic effects. Further analysis of the nature and significance of estrogen and antiestrogen interactions with T3 seems warranted.

References


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