Six Month Oral Toxicity Study of (*E/Z*)-Endoxifen In Rats

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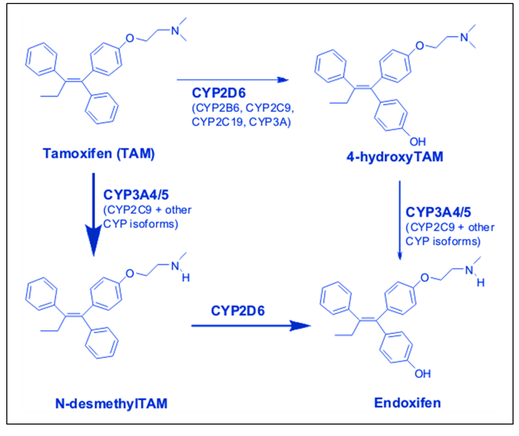
ABSTRACT

The tamoxifen metabolite, endoxifen (N-desmethyl-4-hydroxytamoxifen), is being developed for oral and local topical administration for prevention and therapy of breast cancer. As part of this development, the toxicity of oral endoxifen was evaluated in a chronic (six-month) study in rats. Groups of 25 female CD rats received daily oral (gavage) exposure to endoxifen at doses of 0 (vehicle control), 5, or 50 mg/kg/day for six months. *In vivo* assessments included survival; clinical and physical signs of toxicity; body weight; food consumption; ophthalmology; and clinical pathology (clinical chemistry, hematology, coagulation). Fifteen rats per group were euthanized and necropsied after six months of endoxifen exposure; remaining rats in each group were euthanized and necropsied after a four-week recovery period. All gross lesions and approximately 45 tissues per animal were evaluated microscopically. In addition, bone marrow smears from rats euthanized for the six month necropsy were evaluated for DNA damage (micronucleus assessment). Daily oral administration of endoxifen at 5 or 50 mg/kg/day for six months induced no treatment-related mortality, clinical evidence of toxicity, or effects on ophthalmology. Endoxifen was not genotoxic, as it had no effect on the incidence of bone marrow micronuclei. Both dose levels of endoxifen induced similar suppressions of body weight gain; food consumption was also significantly decreased in both endoxifen groups. Both dose levels of endoxifen induced gross and microscopic changes in hormone -sensitive tissues. Gross pathology was identified at necropsy in the ovary (cysts) and uterus (small) in both endoxifen groups; mean relative ovarian weight was increased and mean relative uterine weight was decreased in endoxifen groups. Microscopic findings were identified in the ovaries (corpora lutea depletion, cysts), uterus (atrophy, endometrial hyperplasia), cervix (mucinous hypertrophy), mammary gland (hyperplasia), and pituitary gland (vacuolation). These changes are consistent with the activity of endoxifen as an endocrine disruptor, and suggest effects on the hypothalamic-pituitary-gonadal axis. After six months of daily oral administration, both dose levels of endoxifen induced significant reductions in body weight gain and food consumption; alterations in the weights of hormone-sensitive organs; and gross and microscopic effects, primarily in hormone-sensitive tissues. Most of the effects of endoxifen observed in this study can be ascribed to its potent antiestrogenic activity, and are interpreted as signs of its pharmacologic action. However, the gross and microscopic changes seen at both dose levels of endoxifen used in this study demonstrate that a No Observed (Adverse) Effect Level (NO[A]EL) for daily oral administration of endoxifen to female rats for six months could not be determined.

BACKGROUND AND INTRODUCTION

Endoxifen (4-hydroxy-N-desmethyltamoxifen; 4-[(1*E/Z*)-1-[4-[2-(methylamino)ethoxy]-phenyl]-2-phenyl-1-buten-1-yl]-phenol) is a metabolite of tamoxifen that is produced by the sequential action of cytochromes P4503A4/5 (CYP3A4/5) and CYP2D6. Two stereoisomers, (*Z*)-endoxifen and (*E*)-endoxifen, are produced during P450-mediated metabolism of tamoxifen; (*Z*)-endoxifen is the more potent of the two isomers.

Figure 1: Metabolism of Tamoxifen to Endoxifen



Endoxifen binds with high affinity to estrogen receptor α (ERα), and demonstrates *in vivo* and *in vitro* antiestrogenic activities that are comparable to that of the well-studied tamoxifen metabolite, 4-hydroxytamoxifen.

Tamoxifen and Endoxifen in Breast Cancer Prevention and Therapy

Tamoxifen is a standard first-line therapy for women with ER+ breast cancer, and is also effective in breast cancer prevention. Tamoxifen is a prodrug that requires CYP-mediated biotransformation to active metabolites; patient genotype (most notably, the presence of CYP2D6 polymorphisms) is an important determinant of tamoxifen efficacy against breast cancer. Data from clinical studies demonstrate that tamoxifen is significantly less active in women harboring CYP2D6 polymorphisms that reduce their ability to metabolize tamoxifen. Similarly, exposure to CYP2D6 inhibitors (e.g., serotonin reuptake inhibitors such as paroxitene) may also reduce tamoxifen activity.

After oral administration of tamoxifen, plasma levels of endoxifen in patients with functional CYP2D6 are as much as 6-fold greater than are plasma levels of 4-hydroxy-tamoxifen. Considering (a) the comparable antiestrogenic potencies of endoxifen and 4-hydroxytamoxifen, and (b) relatively higher plasma levels of endoxifen seen after oral administration of tamoxifen, endoxifen appears to be responsible for much of the pharmacologic activity of tamoxifen.

To obviate reductions in tamoxifen efficacy that may result from reduced metabolism in women with CYP2D6 polymorphisms and/or exposure to CYP2D6 inhibitors, endoxifen is being developed as a potentially more active antiestrogen for breast cancer prevention and therapy.

RATIONALE

This study is a component of a larger preclinical program to characterize the toxicity, pharmacokinetics (PK), and metabolism of endoxifen for breast cancer prevention. The primary focus of the program is the preclinical development of a gel formulation of endoxifen that is designed for direct topical application to the skin of the breast. In comparison to topical administration to the breast, a far greater proportion of an oral dose of endoxifen enters the systemic circulation. This increased systemic exposure to endoxifen results in higher levels of endoxifen reaching potential target tissues and inducing pharmacologic and toxic effects. On this basis, characterization of endoxifen toxicity following repeat-dose oral administration provides a "worst case" scenario for endoxifen toxicity after either its oral administration or its local topical application to the skin of the breast.

Three subchronic oral toxicity studies (in rats, dogs, and minipigs) to characterize the systemic toxicity of endoxifen have been performed in our laboratories. The present study was performed to expand the toxicology database for endoxifen to include its chronic oral administration to rats.

MATERIALS AND METHODS

Animal Welfare: Prior to the initiation of experimentation, the study protocol was reviewed and approved by the IIT Research Institute Animal Care and Use Committee. All work was performed in full compliance with NIH Guidelines for the Care and Use of Laboratory Animals.

Study Design: Three groups of 25 female CD rats each (Table 1, Groups 1 to 3) received oral (gavage) exposure to (*E/Z*)-endoxifen [in a vehicle of 0.03% (w/v) ascorbic acid in sterile water] or vehicle only daily for six months. Fifteen rats per group were necropsied at six months (Main Study); remaining rats were necropsied after a four-week recovery period (Recovery).

Group 4 (5 female CD rats) served as the positive control group for micronucleus studies to evaluate the possible genotoxic activity of endoxifen. One day prior to the Main Study necropsy, all rats in Group 4 received a single intravenous injection of cyclophosphamide (30 mg/kg)

Table 1: Study Design for Six-Month Toxicity Study of Endoxifen in Female Rats

Group No.	Group Identifier	Test or Control Article (Route)	Dose	No. of Rats (Main + Recovery)
1	Vehicle Control	Vehicle (oral)	0 (vehicle control)	15 + 10
2	Low Dose	(*E/Z*)-Endoxifen (oral)	5 mg/kg/day	15 + 10
3	High Dose	(*E/Z*)-Endoxifen (oral)	50 mg/kg/day	15 + 10
4	Genetic Toxicology Positive Control	Cyclophosphamide (intravenous)	30 mg/kg (single dose)	5 + 0

Evaluations and Observations:

In Life (Groups 1 to 3): Mortality/moribundity checks; clinical and physical observations; body weights; food consumption; ophthalmology, clinical pathology (hematology, clinical chemistry, co-agulation, urinalysis);

Post-Mortem (Groups 1 to 3): Organ weights, gross pathology, microscopic pathology

Genetic Toxicology (Groups 1 to 4): micronucleus evaluations on bone marrow samples collect-ed at the Main Study necropsy.

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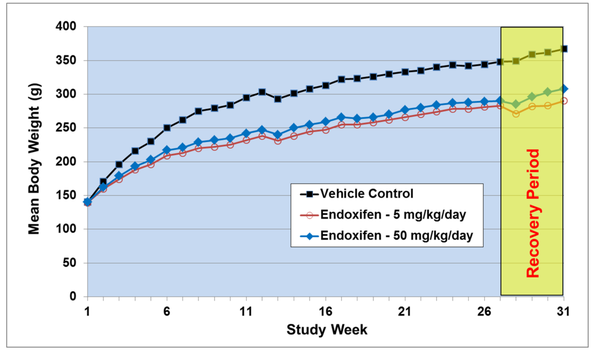
RESULTS

Mortality: No drug-associated mortality was seen during the study.

Clinical and Physical Observations: No gross clinical evidence of endoxifen toxicity.

Body Weights: Statistically significant suppressions of body weight gain in both high and low dose endoxifen groups began during Week 1 and continued through the dosing period (Weeks 1 to 27). Body weight reductions were not reversed during the recovery period (Weeks 27 to 31).

Figure 2: Body Weights



Food Consumption: Consistent with effects on body weight gain, significant decreases in food consumption were seen throughout the dosing period in both groups receiving endoxifen. No differences in food consumption were seen during the recovery period.

Ophthalmology: No ocular changes attributed to endoxifen administration.

Clinical Chemistry: Modest, dose-related increases in ALP in endoxifen-treated rats at Main Study necropsy. Increases in ALP were completely reversed in the low dose group and largely reversed in the high dose group during the recovery period.

Hematology:

- **RBC:** Reversible increases in RBC counts, reticulocyte counts, hematocrit, and total Hb, with apparently compensatory decreases in mean cell volume and mean cell Hb.
- **WBC:** No effects.

Coagulation: Modest increases in PT, APTT, and fibrinogen. After recovery, all parameters in low dose group returned to control; PT and fibrinogen remained elevated in the high dose group.

Urinalysis: Reversible increase in urine pH in both endoxifen groups.

Bone Marrow Micronucleus Evaluation: No significant increase in micronucleus counts in bone marrow erythrocytes in either endoxifen group.

Organ Weights: Because body weights were significantly decreased in endoxifen groups, organ-to-brain weight ratios were compared to identify effects on organ weights (Table 2).

Table 2: Significant, Dose-Related Differences in Organ to Brain Weight Ratios

Organ	Main Study Necropsy		Recovery Necropsy	
	Low Dose	High Dose	Low Dose	High Dose
Heart	↓	↓	↓	↓
Ovaries		↑		↑
Thyroids/Parathyroids		↑		
Uterus	↓	↓	↓	↓

Significant effects on uterine and ovarian weight reflect pharmacologic effects of endoxifen on the hypothalamic/pituitary/gonadal endocrine axis. Reversible effects on thyroid weight may also reflect pharmacologic effects of endoxifen. It has been proposed that antiestrogens modulate thyroid function via cross-talk between estrogen receptors and thyroid hormone receptors

Gross Pathology: Ovarian cysts and small uteri were seen in both endoxifen groups at both necropsies; these correlate with increased ovarian weights and decreased uterine weights.

Microscopic Pathology: Most effects of endoxifen were in hormone-sensitive tissues (Table 3), and are interpreted as secondary to endoxifen action as an antiestrogen/endocrine disruptor.

Table 3: Treatment-related Microscopic Findings

Organ	Main Study Necropsy (n = 15 per group)			Recovery Necropsy (n = 10 per group)		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Ovary: cyst	0	5**	15**	0	2	5**
Ovary: corpus luteum depletion	6	15**	15**	6	10*	8
Uterus: atrophy	1	15**	15**	0	10**	9**
Uterus: hyperplasia	0	5**	0	1	3	4
Cervix: mucinous hypertrophy	0	9**	15**			
Vagina: mucinous hypertrophy	1	15**	15**			
Pituitary: vacuolation	1	8**	14**	0	5**	7**
Lung: alveolar macrophage accumulation	0	0	6**	0	1	1
Mammary Gland: hyperplasia	0	1	8**	0	2	3

*$p < 0.05$ versus control (same necropsy) **$p < 0.01$ versus control (same necropsy)

- Gross findings of ovarian cysts at the Main Study and Recovery necropsies were confirmed microscopically. This effect of endoxifen was partially reversed during the recovery period.
- Gross findings of small uteri at the Main Study and Recovery necropsies were confirmed microscopically; small uteri correlated with varying degrees of uterine atrophy. Uterine atrophy was not reversed during the recovery period.
- Mucinous hypertrophy in the vagina and cervix were common findings in endoxifen-treated rats at the Main Study necropsy. Both effects were reversed during the recovery period.
- Corpus luteum depletion, uterine hyperplasia, and pituitary vacuolation were seen in both endoxifen groups at the Main Study necropsy. These changes were either not reversed or minimally reversed during recovery.
- Dose-related accumulation of alveolar macrophages was identified at lower incidence than were changes in hormone-sensitive tissues. Alveolar macrophage accumulation was largely reversed during the recovery period.
- A dose-related induction of mammary gland hyperplasia was seen in endoxifen-treated rats at the Main Study necropsy. This unexpected finding, which was partially reversed during recovery, is of some concern for the possible use of endoxifen for breast cancer prevention.

CONCLUSIONS

1. Except for reductions in body weight gain that are apparently secondary to pharmacologic (antiestrogenic) activity, chronic oral administration of (*E/Z*)-endoxifen at doses of up to 50 mg/kg/day was well tolerated by female rats.
2. Reductions in body weight gain induced by both doses of endoxifen were seen during the first week of the study and persisted throughout the period of endoxifen administration. Body weight reductions were not reversed during the recovery period.

   Suppression of body weight by antiestrogens appears to be secondary to pharmacologic activity: in preliminary dose-response studies supporting breast cancer chemoprevention studies of tamoxifen (McCormick & Moon, Carcinogenesis, 1986), we were unable to identify pharmacologically active doses of tamoxifen that did not also suppress body weight gain in rats. Even at very low doses, we could not dissociate the effects of tamoxifen on body weight from its antiestrogenic and chemopreventive effects in the mammary gland.

   On this basis, we interpret the observed suppression of body weight gain by endoxifen in this study as secondary to its pharmacologic activity rather than as clear evidence of its toxicity.
3. Modest changes in several clinical pathology parameters were identified in endoxifen-treated rats. These changes were largely reversed during the recovery period, and do not provide evidence of limiting toxicity of the endoxifen doses used in this study.
4. The results of bone marrow micronucleus evaluations identified no evidence of DNA damage induced by endoxifen. The bone marrow micronucleus assay is a standard in vivo test system for preclinical assessment of genetic toxicity.
5. Gross and microscopic pathology induced by endoxifen was seen primarily in hormone-sensitive tissues, particularly those of the reproductive tract. Gross and microscopic changes in the ovary, uterus, cervix, vagina, and pituitary are interpreted as secondary to the pharmacologic activity of chronic administration of endoxifen.
6. An unexpected microscopic finding was the dose-related induction of mammary gland hyperplasia in rats receiving chronic oral exposure to endoxifen. This finding, although largely reversed during the recovery period, should be carefully considered in the use of this drug for breast cancer prevention in otherwise healthy women.
7. Although statistically significant reductions in body weight gain were seen in both groups of female rats receiving chronic oral exposure to endoxifen, no evidence of limiting toxicity was seen. However, in consideration of these body weight effects and the gross and microscopic pathology induced by both dose levels of endoxifen used in this study, a No Observed Effect Level [NOEL] for endoxifen could not be identified.