Repeat-Dose Toxicity and Metabolism of Topical E/Z-Endoxifen Gel in Minipigs

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ABSTRACT

When compared to oral or intravenous administration, direct topical application of breast cancer drugs may increase local delivery and reduce systemic absorption, thereby increasing efficacy and reducing toxicity. A 28-day repeat-dose study was performed in female minipigs to characterize the toxicity and metabolism of an *E/Z*-endoxifen gel being developed for breast cancer prevention. Groups of 8 Göttingen minipigs received topical (dermal) exposure to *E/Z*-endoxifen gel (*E/Z*-endoxifen doses of 2 or 4 mg/day) or vehicle gel only for 28 consecutive days. Gels were applied daily to the same nipple of each minipig; the high dose was the maximum deliverable dose to the nipple. In addition, a pharmacology comparator group of 4 minipigs received daily capsule exposure to *Z*-endoxifen for 28 days. Endpoints included mortality; body weight; clinical observations; local tolerability; clinical pathology; drug and metabolite levels (plasma, mammary gland, liver); organ weights; gross pathology; and microscopic pathology. Topical application of *E/Z*-endoxifen gel was well-tolerated by female minipigs: no mortality, clinical evidence of toxicity, local toxicity, body weight effects, changes in clinical pathology parameters, or gross or microscopic findings were observed. Dose-related decreases in absolute and relative ovary weights were seen in both groups receiving topical *E/Z*-endoxifen gel, reflecting the pharmacologic action of this drug. Endoxifen is rapidly glucuronidated in minipigs; with few exceptions, plasma levels of endoxifen were below the limit of quantitation, regardless of route of exposure. Endoxifen and endoxifen glucuronide were measurable in the mammary gland (but not plasma or liver) of minipigs receiving topical E/Z-endoxifen gel. By contrast, high levels of endoxifen glucuronide were present in the plasma of minipigs receiving oral exposure to Z-endoxifen. Both endoxifen and endoxifen glucuronide were measurable in the liver of minipigs receiving oral exposure; endoxifen glucuronide was also present in the mammary glands from the oral exposure group. Repeat-dose topical administration of *E/Z*-endoxifen gel for 28 days is well-tolerated in female minipigs, and provides higher tissue levels of endoxifen than administration of a much higher oral dose of *Z*-endoxifen.

INTRODUCTION

Endoxifen (4-hydroxy-N-desmethyltamoxifen) is a tamoxifen metabolite produced by sequential action of cytochromes P4503A4 (CYP3A4) and CYP2D6. As a result of its high affinity binding to estrogen receptor α (ERα), endoxifen demonstrates antiestrogenic activity comparable to that of the well-studied tamoxifen metabolite, 4-hydroxytamoxifen. Two stereoisomers of endoxifen, (*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. Structure of (*Z*)-endoxifen

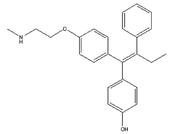
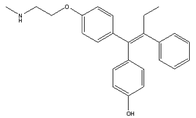


Figure 2. Structure of (*E*)-endoxifen



Tamoxifen and Endoxifen in Breast Cancer Prevention and Therapy

Tamoxifen is a standard first-line therapy for women with ER+ breast cancer. Clinical data also demonstrate tamoxifen efficacy in breast cancer prevention. However, patient genotype (particularly the presence of CYP2D6 polymorphisms) is an important determinant of tamoxifen efficacy against breast cancer: clinical studies demonstrate that tamoxifen is significantly less active in women with CYP2D6 polymorphisms that reduce their ability to metabolize tamoxifen. Concomitant exposure to inhibitors of CYP2D6 (e.g., serotonin reuptake inhibitors such as paroxitene) may also reduce tamoxifen activity. On this basis, tamoxifen may be considered to be a prodrug that requires CYP-mediated biotransformation to one or more active metabolites.

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

To obviate reductions in tamoxifen efficacy that may result from its reduced metabolism in women with CYP2D6 polymorphisms and/or exposure to CYP2D6 inhibitors, (*E/Z*)-endoxifen, a mixture of the two geometric isomers of endoxifen, is being developed for possible use in breast cancer prevention and therapy.

Transdermal SERMS in Breast Cancer Prevention and Therapy

Recent evidence suggests that administration of tamoxifen or other selective estrogen receptor modulators (SERMs) by direct topical application to the breast may both increase efficacy and decrease systemic toxicity.

- In a randomized Phase II presurgical trial of women with ductal carcinoma *in situ*, topical administration of gel containing 4-hydroxytamoxifen to the skin of the breast demonstrated antiproliferative effects in the breast that were similar to those seen with oral administration of tamoxifen. Importantly, local topical administration of 4-hydroxytamoxifen induced less systemic toxicity than did oral administration.
- Preclinical studies demonstrate that levels of endoxifen in the mammary gland are significantly higher in rats receiving local topical administration of an endoxifen gel than in rats receiving oral exposure to tamoxifen.

These preclinical and clinical studies provide proof of concept that local application of tamoxifen metabolites to the skin of the breast can generate higher levels of these active metabolites in the breast while reducing systemic toxicity resulting from oral administration of the prodrug, tamoxifen.

RATIONALE

This study is a component of a preclinical program to characterize the toxicity, pharmacokinetics (PK), and metabolism of a gel formulation of endoxifen designed for topical application to the skin of the breast. It is proposed that local delivery of endoxifen will (a) maximize the quantity of endoxifen that reaches the breast epithelium, thereby optimizing endoxifen activity; and (b) reduce or eliminate potential systemic toxicities that may result from oral drug administration.

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STUDY GOALS

1. Assess the local toxicity and tolerability of repeat-dose topical application of endoxifen gel to the nipple area overlying the mammary gland of the minipig.
2. Characterize the systemic toxicity of repeat-dose topical application of endoxifen gel in the minipig.
3. Quantitate levels of endoxifen and major metabolites in the plasma, mammary gland, and liver of minipigs receiving repeat-dose topical administration of endoxifen gel.

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 8 female minipigs each (Table 1, Groups 1 to 3) received daily topical exposure to *E/Z*-endoxifen gel or vehicle gel for 28 consecutive days. Endoxifen gel and vehicle gel were administered daily to the same site in each minipig (a ~2 cm^2 area surrounding the same nipple). A pharmacology comparator group consisting of 4 minipigs (Table 1, Group 4) received daily oral (capsule) exposure to *Z*-endoxifen for 28 days.

Table 1. Study Design: 28-Day Toxicity Study of (E/Z)-Endoxifen Gel in Female Minipigs

Group No.	Group Identifier	Test or Control Article (Route)	Dose and Vehicle[a]	No. of Minipigs (Main + Recovery)
1	Vehicle Control (Toxicology)	Vehicle Gel (Topical)	0 (control) in 400 µL gel	4 + 4
2	Low Dose (Toxicology)	*E/Z*-Endoxifen Gel (Topical)	2 mg endoxifen in 400 µL gel	4 + 4
3	High Dose (Toxicology)	*E/Z*-Endoxifen Gel (Topical)	4 mg endoxifen in 400 µL gel	4 + 4
4	Pharmacology Comparator	*Z*-Endoxifen (Oral)	1 mg/kg (oral; capsule)	4 + 0

[a] 400 µL is the maximum deliverable volume to ~2 cm^2 area centered on the nipple.

Parameter	Description
Experimental Animals	Female Göttingen minipigs (aged 6 to 7 months) from Marshall BioResources, North Rose, NY. Minipigs were quarantined for 4 weeks prior to study start.
Animal Husbandry	
Housing	Individually in floor-level runs
Basal Diet	Certified Miniswine Diet #7037C (Harlan/Teklad, Madison, WI)
Drinking Water	City of Chicago, supplied by automatic watering system
Animal Room Light Cycle	12 hours light/12 hours dark per day
Test and Control Articles	Vehicle gel (0% endoxifen), *E/Z*-endoxifen gels containing 0.5% (low dose) or 1.0% (high dose) *E/Z*-endoxifen, and bulk *Z*-endoxifen (for oral administration) were supplied with Certificates of Analysis by the National Cancer Institute. Test and control articles were used without further purification.
Test and Control Article Storage and Administration	Test and control articles were stored at controlled room temperature. Gel formulations were administered topically at a volume of 400 µL per dose. In a preliminary feasibility study, 400 µL was determined to be the maximum volume of gel that could be delivered to a 2 cm^2 area centered on the nipple of the minipig. Z-endoxifen was administered orally (by capsule).
Evaluations and Observations (Groups 1 to 3)	
Mortality/Moribundity Checks	Twice daily
Clinical Observations	At least once daily during the dosing and recovery periods
Body Weights	Weekly during the dosing and recovery periods
Food Consumption	Daily during the dosing and recovery periods
Local Tolerability (Draize scoring)	At approximately 2 hours post-dosing on Days 1 to 7 and on Days 14, 21, and 28
Clinical Pathology (Hematology, Clinical Chemistry, Coagulation)	Pretest; Days 15 and 28 (dosing period); Day 42 (recovery)
Plasma Drug Levels	Plasma samples were collected at predose and at 1, 2, 4, 8, 12, and 24 hours after dosing on Days 1, 11, and 27. Plasma levels of endoxifen and endoxifen glucuronide were quantitated by LC/MS-MS.
Tissue Drug Levels	Samples of mammary gland and liver collected at the terminal necropsy of pharmacology comparator animals. Tissue levels of endoxifen and endoxifen glucuronide were quantitated by LC/MS-MS, as described above.
Organ Weights	Collected at Main Study + Recovery necropsies; brain, uterus + cervix (weighed together), adrenals (paired), heart, kidneys (paired), liver, ovaries (paired), spleen, thymus, thyroids (paired)
Gross Pathology	Complete necropsy with organ weights and tissue collection, all animals
Microscopic Pathology	Microscopic evaluation of all tissues from all animals
Statistical Evaluations	Continuous data were compared via analysis of variance, with *post-hoc* comparisons made using Dunnett's test. Incidence comparisons were compared using X^2 analysis. A minimum significant level of $p < 0.05$ was used in all comparisons.

RESULTS

Toxicology

Clinical Parameter	Observation
Mortality	No mortality was seen during the study.
Clinical Observations	No gross clinical evidence of endoxifen toxicity was identified in any study animal.
Body Weights and Food Consumption	Body weights and food consumption in endoxifen-treated minipigs were comparable to those of vehicle controls at all times in the study.
Local Tolerability/Dermal Irritation	No evidence of edema, erythema, or eschar at the site of application was identified in any minipig receiving daily topical exposure to *E/Z*-endoxifen gel.
Clinical Pathology	No statistically significant, treatment-related changes in any hematology, clinical chemistry, or coagulation endpoint were identified in any animal exposed to endoxifen.
Organ Weights	Statistically significant, dose-related decreases in absolute and relative ovary weights were seen in both groups receiving daily topical exposure to *E/Z*-endoxifen gel. These changes are interpreted as reflecting the pharmacologic action of endoxifen. No other statistically significant differences in organ weights were seen.
Gross Pathology	No evidence of gross pathology was identified in any minipig receiving daily topical exposure to *E/Z*-endoxifen gel.
Microscopic Pathology	No microscopic evidence of endoxifen toxicity was identified in any study animal receiving daily topical exposure to *E/Z*-endoxifen gel.
Endoxifen Plasma Levels	
Topical Administration Groups	Plasma levels of endoxifen were below the LOQ (0.2 ng/mL) in most samples collected from minipigs receiving daily topical exposure to *E/Z*-endoxifen gel. Endoxifen was measurable only at trace levels in a few specimens on each collection day.
Oral Administration Group	On Study Day 1, plasma levels of endoxifen were below the LOQ (0.2 ng/mL) in minipigs receiving oral administration of endoxifen. However, plasma levels of endoxifen were above the LOQ in approximately half of samples collected on Study Day 11 and in most samples collected on Study Day 27. When above the LOQ, plasma concentrations of endoxifen were generally < 0.5 ng/mL.
Endoxifen Glucuronide Plasma Levels	
Topical Administration Groups	Plasma levels of endoxifen glucuronide were below the LOQ in most samples collected on Study Days 1, 11, and 27. When plasma levels were above the LOQ, endoxifen glucuronide was detected only at trace levels (<0.5 ng/mL). These results are consistent with the lack of measurable plasma levels of *Z*-endoxifen in most minipigs receiving daily topical exposure to *E/Z*-endoxifen gel.
Oral Administration Group	Plasma levels of endoxifen glucuronide in minipigs receiving daily oral exposure to *Z*-endoxifen increased throughout the dosing period, and were substantially higher than plasma levels in groups dosed topically with endoxifen gel. Plasma Cmax values for endoxifen glucuronide in minipigs receiving oral administration of *Z*-endoxifen were 74 ng/mL, 174 ng/mL, and 446 ng/ml on Study Days 1, 11, and 27, respectively. The >500-fold difference in plasma levels of endoxifen glucuronide versus endoxifen in minipigs receiving oral exposure to *Z*-endoxifen demonstrate the rapid and nearly complete conversion of parent drug to its glucuronide metabolite.
Levels of Endoxifen and Endoxifen Glucuronide in the Mammary Gland	
Topical Administration Groups	Levels of endoxifen measured in the mammary glands of minipigs receiving local topical exposure to *E/Z*-endoxifen gel increased with dose (71 µg/g and 380 µg/g in the low and high dose groups, respectively).
	Levels of endoxifen glucuronide measured in the mammary gland were much lower than levels of endoxifen measured in the same samples. Two possible explanations can be identified to explain this finding. The higher levels of parent drug (versus glucuronide) may reflect the relative lack of drug metabolizing activity in the mammary parenchyma (versus, for example, the liver). Alternatively, however, possible contamination of mammary gland samples from residual test article remaining at the dosing site cannot be excluded as a possible source of parent compound measured in the mammary gland.
Oral Administration Group	Mean concentrations of endoxifen and endoxifen glucuronide in the mammary gland after oral administration were 0.14 µg/g and 0.11 µg/g, respectively. The similar concentrations of both the parent drug and metabolite in the mammary gland following oral administration support the argument made above that only limited metabolism of endoxifen occurs in the mammary gland.
Levels of Endoxifen and Endoxifen Glucuronide in the Liver	
Topical Administration Groups	Hepatic levels of endoxifen were below the LOQ in both groups receiving topical exposure to *E/Z*-endoxifen gel. Hepatic levels of endoxifen glucuronide were below the LOQ in minipigs receiving the low dose of *E/Z*-endoxifen gel, but were 0.1 µg/g in minipigs receiving the high dose of *E/Z*-endoxifen gel.
Oral Administration Group	Mean concentrations of (*Z*)-endoxifen and endoxifen glucuronide in the liver after oral administration were 0.64 µg/g and 32 µg/g, respectively, demonstrating extensive hepatic or other systemic metabolism of *Z*-endoxifen.

CONCLUSIONS

- Topical administration of a gel formulation containing *E/Z*-endoxifen was very well tolerated by female Göttingen minipigs. Daily administration of endoxifen doses of 2 mg or 4 mg/day for 28 consecutive days induced no evidence of local irritation or systemic toxicity in any study animal. Body weights, food consumption, clinical observations, and hematology, clinical chemistry, and coagulation parameters in endoxifen-treated rats were comparable to those in vehicle-treated controls.
- Gross pathology at the terminal necropsy and microscopic evaluation of tissues also failed to identify any pattern of treatment-related toxicity resulting from repeat-dose topical administration of endoxifen. The only statistically significant finding in endoxifen-treated groups at the terminal necropsy was a dose-related reduction in absolute and relative ovary weights; these effects are ascribed to the pharmacologic activity of the drug.
- Endoxifen is rapidly and completely glucuronidated after systemic administration. Plasma levels of both endoxifen and endoxifen glucuronide were very low in groups receiving daily topical exposure to endoxifen gel, and were often below the LOQ (0.2 ng/mL). Although plasma levels of endoxifen were also low in minipigs receiving daily oral administration of endoxifen (pharmacology comparator group), levels of endoxifen glucuronide in animals receiving oral endoxifen increased with increasing duration of exposure.
- Endoxifen levels in the mammary gland of minipigs receiving daily topical administration of endoxifen gel were substantially greater than were mammary gland levels of endoxifen glucuronide. Endoxifen levels in the mammary gland of minipigs receiving oral endoxifen exposure were much lower than in animals receiving topical exposure; endoxifen levels in the mammary gland of orally-exposed minipigs were comparable to mammary gland levels of endoxifen glucuronide.