

# Endoxifen, a New Cornerstone of Breast Cancer Therapy: Demonstration of Safety, Tolerability, and Systemic Bioavailability in Healthy Human Subjects

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Endoxifen is an active metabolite of tamoxifen, a drug used in the treatment of breast cancer. In order to be clinically effective, tamoxifen must be converted to endoxifen by cytochrome P450 2D6 (CYP2D6). A study involving single escalating oral doses was conducted in humans to evaluate the safety, tolerability, and pharmacokinetics (PK) of endoxifen. This is the first study demonstrating that single oral doses of endoxifen are safe and well tolerated and have sufficient bioavailability to reach systemically effective levels in human subjects. Furthermore, it was found that endoxifen is rapidly absorbed and systemically available and that it displays dose proportionality in peak drug concentrations in plasma ( $C_{\max}$ ) and area under the concentration–time curve extrapolated from 0 to  $\infty$  ( $AUC_{0-\infty}$ ) over the dose range 0.5–4.0 mg.

Tamoxifen, when orally administered, is extensively metabolized by cytochrome P450 (CYP) enzymes into active metabolites, including 4-hydroxy tamoxifen and endoxifen (4-hydroxy *N*-desmethyl tamoxifen) (Figure 1). Endoxifen is an active metabolite of tamoxifen, which is widely marketed as a selective estrogen receptor (ER) modulator for use in the treatment of ER-positive or progesterone receptor-positive breast cancer, and is the only drug approved for the prevention of the disease.<sup>1</sup> Endoxifen is 100 times more potent than tamoxifen<sup>2</sup> and is generated through CYP3A4/5-mediated *N*-demethylation and CYP2D6-mediated hydroxylation.<sup>3–8</sup> The genetic polymorphism of CYP2D6 results in large interpatient variations in both therapeutic efficacy and side effects of tamoxifen.<sup>9–15</sup>

Recent reports indicate that coadministration of antidepressants can significantly affect survival in women receiving tamoxifen for breast cancer because of drug–drug interactions.<sup>16</sup> For example, CYP2D6 is inhibited by specific selective serotonin reuptake inhibitors that are frequently used to prevent the hot flushes experienced by up to 45% of patients taking tamoxifen. As a consequence, significant numbers of women might not receive optimal benefit from tamoxifen treatment. This observation is consistent with the critical role of CYP2D6

in the metabolic activation of tamoxifen and highlights a drug interaction that is extremely common, widely underappreciated, and uniformly avoidable.

Because tamoxifen is a crucial element of the treatment regimen for patients with hormone receptor-positive breast cancer, regardless of age or breast cancer stage, we sought to evaluate the use of endoxifen as an improved chemical entity that would not be subject to pharmacogenetic variations or drug interactions that affect the activity of CYP2D6. We initially conducted pre-clinical studies to demonstrate the potential anticancer activity of endoxifen in an NCI-60 cell line, human mammary tumor xenografts in female mice, and evaluated its bioavailability and safety in female rats.<sup>17</sup> This report extends these observations by describing, for the first time, the safety, tolerability, and pharmacokinetics (PK) of endoxifen after administration of escalating single oral doses to human subjects.

## RESULTS

### Safety and tolerability evaluation

There were no serious or significant adverse events during the entire course of the study. All the subjects were evaluated with respect to hematologic, biochemical, and serologic laboratory

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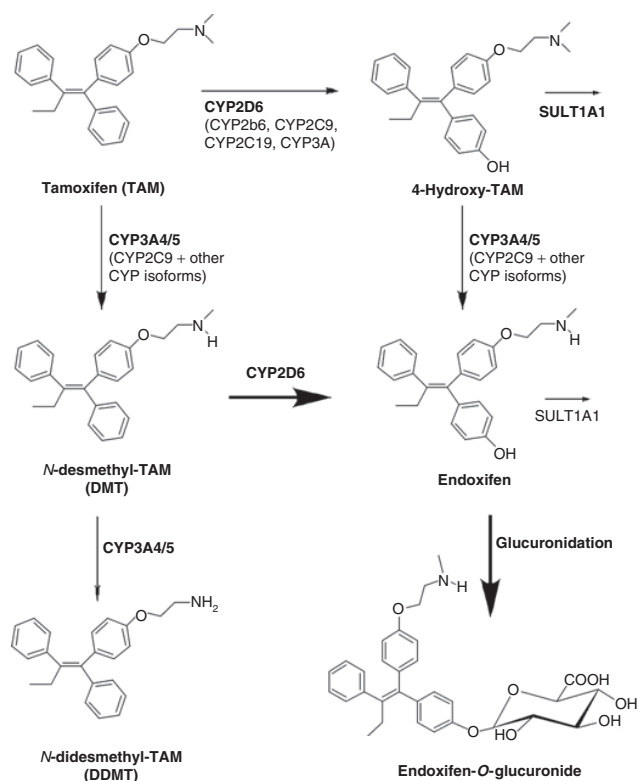
parameters and urine analysis at various time points (prior to enrollment; at 12, 24, and 96 h after the dose; at day 13; and at the end of study). All the laboratory parameters were measured in accordance with accepted standard operating procedures, and the results were reviewed by a pathologist. None of the subjects had any clinically significant abnormalities during the conduct of the study. The vital signs recorded in all the subjects at various time points (after check-in; at 1, 3, 6, 12, 24, 36, 48, and 84 h after the dose; before checkout; on day 13; and at the end of study, after the last ambulatory sample) were found to be within normal limits. Electrocardiograms performed at 1, 3, and 96 h after the dose; on day 13; and at the time of checkout were found to be within normal limits. One adverse event (diarrhea) was reported by a subject (1 of the 32 subjects, i.e., 3.13%) who had received 0.5 mg of endoxifen during the

study, but it was mild and judged to be “not related” to the administration of endoxifen.

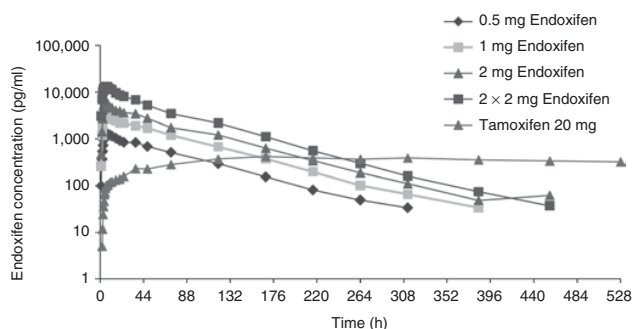
### Pharmacokinetics

Concentration-vs.-time profiles for endoxifen administered at different single doses and for endoxifen formed from a single dose of tamoxifen are shown in **Figure 2**. Endoxifen administered as a mono-entity is absorbed faster and is mostly eliminated from plasma by 264 h after the dose. However, endoxifen formed in the body after metabolism of orally administered tamoxifen took a considerably longer time (~168 h) to reach peak levels and then declined with an apparent half-life of 1,051 h (confidence interval 608–1,495), reflecting tamoxifen's long elimination half-life rather than the half-life of endoxifen itself (“flip-flop” kinetics).

**Table 1** lists the PK parameters of endoxifen in humans. Regression and correlation analyses of peak drug concentrations in plasma ( $C_{\max}$ ) and area under the concentration–time curve extrapolated from 0 to  $\infty$  ( $AUC_{0-\infty}$ ) by dose indicated that the relationship was linear with  $r^2 = 0.9946$  for  $C_{\max}$  and  $r^2 = 0.9965$  for  $AUC_{0-\infty}$ . This indicates that both rate and extent of absorption of endoxifen increased linearly and in a manner proportional to the dose of endoxifen (tablets of 0.5, 1, 2, and 4 mg). At these doses, the time to peak ( $T_{\max}$ ) values were between 4.5 and 6 h, and half-life ( $t_{1/2}$ ) values were 52.1–58.1 h. The increase in  $C_{\max}$  and AUC appeared to be proportional to dose when consecutive dose increments were compared (**Figure 3a,b**). Endoxifen administered at oral doses of 0.5, 1, 2, or 4 mg resulted in  $C_{\max}$  values of 1.38, 3.98, 6.79, and



**Figure 1** Metabolism of tamoxifen by cytochrome P450 (CYP).



**Figure 2** Semilogarithmic plots of mean concentrations of endoxifen in plasma vs. time after the indicated single doses of endoxifen or tamoxifen.

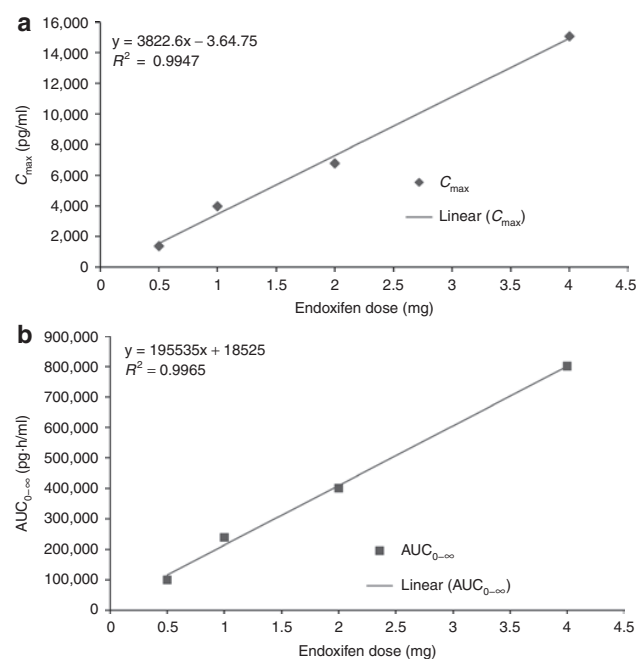
**Table 1** Endoxifen doses and pharmacokinetic parameters

Dose	$C_{\max}$ (ng/ml)	$AUC_{0-\infty}$ (ng·h/ml)	$t_{1/2}$ (h (CV%))	Vz (l)	Cl (l/h)
Endoxifen 0.5 mg	1.38 ± 0.25	99.9 ± 13.6	58.11 (18.0)	427 ± 101	5.1 ± 0.7
Endoxifen 1.0 mg	3.98 ± 1.7	239 ± 70	54.1 (10.6)	346 ± 88	4.5 ± 1.1
Endoxifen 2.0 mg	6.79 ± 1.85	401 ± 113	55.4 (16.3)	428 ± 133	5.4 ± 1.8
Endoxifen 4.0 mg	15.1 ± 4.24	801 ± 262	52.1 (12.9)	406 ± 119	5.5 ± 1.9
Tamoxifen 20 mg	0.417 ± 0.013	381 ± 47.6	1,051 (16.4) <sup>a</sup>	Fixed	Fixed

Data are given as mean values ± SD except for  $t_{1/2}$  (coefficient of variation percentage);  $n = 8$  subjects/treatment group. Fixed—could not be estimated from data for tamoxifen, and therefore, values fixed at  $V_z = 400$  l and  $Cl = 5.0$  l/h.

$AUC_{0-\infty}$ , area under the concentration–time curve extrapolated from 0 to  $\infty$ ;  $C_{\max}$ , peak drug concentrations in plasma; Cl, confidence interval; CV, coefficient of variation;  $t_{1/2}$ , half-life.

<sup>a</sup>Apparent  $t_{1/2}$  estimated from terminal exponential phase of the concentration-vs.-time curve.



**Figure 3** Regression analysis showing (a) proportionality between dose and  $C_{max}$  and (b) proportionality between dose and  $AUC_{0-\infty}$ .  $AUC_{0-\infty}$ , area under the concentration–time curve extrapolated from 0 to  $\infty$ ;  $C_{max}$ , peak drug concentrations in plasma.

15.1 ng/ml, respectively, and  $AUC_{0-\infty}$  values of 100, 239, 401, and 801 ng-h/ml, respectively. When compared with endoxifen levels ( $C_{max}$ , 0.417 ng/ml) in subjects who received 20 mg tamoxifen (Nolvadex), 0.5, 1, 2, and 4 mg endoxifen showed 231, 854, 1,528, and 3,521% higher  $C_{max}$  values, respectively. The  $C_{max}$  was achieved faster with the administration of endoxifen than with tamoxifen.

The mean terminal elimination ( $t_{1/2}$ ) of endoxifen after a single dose of 4 mg was 52.05 h, and the elimination rate constant ( $k_e$ ) was 0.0133/h. The calculated accumulation index value was 3.65. Using these values, the estimated steady-state plasma concentration ( $C_{max}^{ss}$ ) of endoxifen is 55.1 ng/ml when the drug is administered in multiple doses of 4 mg at dose intervals of 24 h (see Methods section). The endoxifen  $V_z$  and  $Cl$  values after administration of tamoxifen could not be estimated from the concentration values in plasma shown in Figure 2. However,  $AUC_{0-\infty}$  was 381 ng-h/ml when endoxifen  $V_z$  and  $Cl$  were assumed to be similar to results obtained when endoxifen was administered (400 l and 5 l/h, respectively).

## DISCUSSION

Tamoxifen is widely used for the prevention and treatment of premenopausal and postmenopausal patients with ER-positive breast cancer. It is a prodrug that is extensively metabolized and converted to potent antiestrogens. Tamoxifen and its metabolites shown in Figure 1—4-hydroxytamoxifen, *N*-desmethyltamoxifen, didesmethyltamoxifen, and endoxifen—are selective ER modulators. The major route of tamoxifen metabolism in humans is demethylation, yielding

*N*-desmethyltamoxifen and didesmethyltamoxifen. However, *N*-desmethyltamoxifen and didesmethyltamoxifen have lower affinities for ER, whereas endoxifen and 4-hydroxytamoxifen concentrations, although low, are more potent and have a higher affinity for ER.<sup>2,3</sup>

In patients taking tamoxifen at the prescribed dosage of 20 mg/day, the mean steady-state concentrations of tamoxifen, 4-hydroxytamoxifen, and *N*-desmethyltamoxifen are 335, 7.4, and 695 nmol/l, respectively.<sup>5</sup> However, the concentrations of endoxifen in plasma vary widely and are dependent on the activity of CYP2D6. The CYP2D6 phenotypes that result from genetic polymorphisms include poor metabolizers, intermediate metabolizers, extensive metabolizers, and ultrarapid metabolizers. Among the patients on tamoxifen, those who are poor metabolizers and have the CYP2D6 \*4/\*4 genotype tend to have a higher risk of disease relapse and a lower incidence of hot flashes.<sup>9</sup> Kiyotani *et al.*<sup>12</sup> also found that the lower clinical efficacy of tamoxifen in poor metabolizer patients with CYP2D6 \*10/\*10 may be caused by lower systemic exposure to endoxifen. The median steady-state plasma concentrations of endoxifen found in Japanese (10) and Caucasian (7) patients with genotype wt/wt were 35.4 and 52.3 ng/ml, respectively. These women were taking 20 mg of tamoxifen per day. The concentration of endoxifen in the range of 35.4–52.3 ng/ml can easily be achieved by oral administration of an endoxifen dose of 4.0 mg.

In our study, we observed that endoxifen is rapidly absorbed and displays dose-proportional PK with respect to  $C_{max}$  and  $AUC_{0-\infty}$ . After a single dose of 4 mg, a  $C_{max}$  of 15.1 ng/ml was observed. Therefore, in the multiple-dose study, the  $C_{max}^{ss}$  at steady state ( $C_{max} \times$  accumulation index,  $15.1 \times 3.65$ ) with a 4-mg once-daily dosage is expected to be 55.1 ng/ml. On the basis of these results, we expect that multiple daily endoxifen doses of 2.0–4.0 mg will result in endoxifen exposures that would be similar to those found in patients with normal CYP2D6 function who are administered tamoxifen at 20 mg/day. That is, a dose of 4 mg of endoxifen should be appropriate for breast cancer prevention and therapy.

In summary, we propose that substitution of endoxifen for tamoxifen will provide an improved approach toward treating patients with breast cancer because it bypasses the CYP2D6 enzyme that is required for metabolic activation of tamoxifen. Consequently, its activity is not likely to be affected by either CYP2D6 genetic polymorphisms or drug–drug interactions that inhibit CYP2D6 activity. This is a first-in-human study to evaluate the safety, bioavailability, and PK of endoxifen.

## METHODS

**Endoxifen synthesis.** Synthesis of endoxifen citrate was carried out as described previously in multiple steps starting from 4-bromophenol.<sup>18</sup> Endoxifen citrate was prepared as an enteric-coated tablet for oral administration to human subjects. Enteric-coated tablets (0.5, 1.0, 2.0, or 4 mg (two tablets of 2 mg)) of endoxifen citrate were manufactured in accordance with current good manufacturing practices.

**Study design and dose levels.** Forty human subjects of Asian Indian origin were randomized to receive either endoxifen or tamoxifen as follows: a total of 32 subjects received single oral doses of endoxifen,

with 8 subjects in each of four dose subgroups (0.5, 1, 2, and 4 mg). The other eight subjects in the study received a single dose each of 20 mg of tamoxifen (2 × 10-mg Novaldex tablets). The subjects were not tested for CYP2D6 and CYP2C19 genotypic variations. All the subjects fasted overnight for at least 10 h before the study dose was administered, and all the drug doses were administered orally while the subjects were in a seated posture.

**Blood sampling, plasma collection, and assay.** Blood samples were collected for PK study before the dose and at various time points, up to 528 h, after the dose. Plasma was separated by centrifuging the blood samples at 3,000 relative centrifugal force for 5 min at <10 °C. The separated plasma was stored at −55 °C until completion of the analysis. A liquid chromatography–tandem mass spectrometry method (previously described) was used to quantify endoxifen concentrations in the plasma samples, with anastrozole as the internal standard.<sup>17</sup> The samples were chromatographed on a Kromasil 100 C8 5 μm 150 × 4.6 mm column using an isocratic mobile phase system composed of acetonitrile and formic acid solution. Endoxifen and the internal standard were monitored in the positive-ion mode using the multiple-reaction monitoring transitions. Analyst software (version 1.4.1; Applied Biosystems, Foster City, CA) was used for the evaluation of chromatographic data.

**PK analysis.** Standard PK parameters were calculated from the drug concentration–time profile by noncompartmental methods using WinNonlin Professional software (version 5.0.1; Pharsight, Mountain View, CA). Estimates of the PK parameters of endoxifen formed from tamoxifen were obtained using the SAAM II program (SAAM Institute, Seattle, WA).

The accumulation index ( $R_{AC}$ ) was applied to predict the maximal concentration in plasma during steady state from single-dose data.<sup>19</sup>

$$C_{\max}^{ss} = C_{\max} \times R_{AC}$$

$R_{AC}$  is calculated from  $1/(1 - \exp(-k_e \cdot \tau))$ , where  $k_e$  is the elimination rate constant obtained from the equation  $k_e = 0.693/t_{1/2}$ , and  $\tau$  is the dose interval.

**Clinical safety measurements.** All subjects were clinically examined, and vital signs (blood pressure, radial pulse, respiratory rate, and oral body temperature) were recorded at check-in; at 1, 3, 6, 12, 24, 36, 48, and 84 h after the dose; before checkout; on day 13 of dosing; and at the end of the study. Hematology; biochemistry; immunological function; serum electrolyte, cholesterol, and triglyceride levels; and urine assessments were performed at check-in; at 12, 24, and 96 h; on day 13; and at the end of the study.

**Statistics.** Individual values, mean values, and SD of  $C_{\max}$ ,  $T_{\max}$ , and  $AUC_{0-\infty}$  were calculated for endoxifen. The relationship between the primary mean PK parameters ( $C_{\max}$  and  $AUC_{0-\infty}$ ) for different doses of endoxifen was interpreted using regression and correlation analysis and carried out using PROC MIXED of SAS release 9.1.3 (SAS Institute, Cary, NC).

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#### CONFLICT OF INTEREST

All the authors are current employees of Jina Pharmaceuticals (Libertyville, IL), Intas (Ahmedabad, India), or Lambda Therapeutic Research (Ahmedabad, India).

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