Clinical Cancer Research

A Randomized Phase II Presurgical Trial of Transdermal 4-Hydroxytamoxifen Gel versus Oral Tamoxifen in Women with Ductal Carcinoma *In Situ* of the Breast

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Abstract

Purpose: Local transdermal therapy to the breast may achieve effective target-organ drug delivery, while diminishing systemic effects. We conducted a randomized, double-blind, placebo-controlled phase II trial comparing transdermal 4-hydroxytamoxifen gel (4-OHT) to oral tamoxifen (oral-T) in women with ductal carcinoma *in situ* (DCIS).

Methods: Twenty-seven pre- and postmenopausal women were randomized to 4-OHT (4 mg/day) or oral-T (20 mg/day) for 6 to 10 weeks before surgery. Plasma, nipple aspirate fluid, and breast adipose tissue concentrations of tamoxifen and its major metabolites were determined by liquid chromatography/tandem mass spectrometry. The primary endpoint was Ki67 labeling in DCIS lesions, measured by immunohistochemistry. In plasma, insulin-like growth factor-1 (IGFI), sex hormone–binding globulin (SHBG), and coagulation protein concentrations were determined.

Results: Posttherapy Ki67 decreased by 3.4% in the 4-OHT and 5.1% in the oral-T group ($P \leq 0.03$ in both, between-group $P = 0.99$). Mean plasma 4-OHT was 0.2 and 1.1 ng/mL in 4-OHT and oral groups, respectively ($P = 0.0003$), whereas mean breast adipose tissue concentrations of 4-OHT were 5.8 ng/g in the 4-OHT group and 5.4 ng/g in the oral group ($P = 0.88$). There were significant increases in plasma SHBG, factor VIII, and von Willebrand factor and a significant decrease in plasma IGFI with oral-T, but not with 4-OHT. The incidence of hot flashes was similar in both groups.

Conclusions: The antiproliferative effect of 4-OHT gel applied to breast skin was similar to that of oral-T, but effects on endocrine and coagulation parameters were reduced. These findings support the further evaluation of local transdermal therapy for DCIS and breast cancer prevention. *Clin Cancer Res; 20(14); 3672–82. ©2014 AACR.*

Introduction

Mammary ductal carcinoma *in situ* (DCIS) accounts for 20% of new breast cancers (1), with 57,000 new cases diagnosed in the United States in 2011 (2). Although disease-specific survival rates approach 98% (3), the risk for the development of subsequent invasive breast cancer may reach 30% following local therapy (4), so that patients with DCIS are advised to undertake systemic therapy in the form of oral tamoxifen (oral-T) in order to further reduce the risk of new (local) breast events.

Despite the success of tamoxifen in reducing recurrence risk of estrogen receptor (ER)–positive DCIS and that of new breast primaries (5, 6), its systemic effects have led to generally low acceptance in the DCIS and prevention setting (4–8). These relate to estrogen agonist activity on the endometrium and the activation of coagulation pathways, leading to an increased risk of uterine events and thromboembolism (9). Hot flashes and vaginal symptoms are an additional barrier to acceptance (7, 10). Thus, a particular challenge for primary and secondary breast cancer prevention efforts is to devise an efficacious and nontoxic intervention, which is likely to be widely accepted by women who will benefit from it.

One possible solution is transdermal delivery of active drugs through the skin envelope of the breast to achieve

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Translational Relevance

Women at high risk for breast cancer and those with ductal carcinoma *in situ* (DCIS) are reluctant to accept oral (i.e., systemic) therapy with tamoxifen. This is a major barrier to the implementation of pharmacologic prevention strategies. A possible solution is local transdermal therapy (LTT), applying active drug metabolites to the breast skin. One such candidate is 4-hydroxytamoxifen (4-OHT), a potent anti-estrogenic metabolite of tamoxifen. We conducted a randomized, double-blind, presurgical phase II trial comparing transdermal 4-OHT gel to oral tamoxifen (oral-T) in women with DCIS. We observed equivalent antiproliferative effect of transdermal 4-OHT gel and oral-T, but systemic effects on endocrine and coagulation parameters were reduced with transdermal delivery. Furthermore, plasma concentrations of 4-OHT in the gel group were one fifth of those in the oral group, but breast adipose tissue concentrations were similar. These findings support the further evaluation of LTT for DCIS therapy and breast cancer prevention.

high local concentrations with low systemic exposure, exploiting the embryological origins of the breast as a skin appendage (a modified eccrine gland) with a well-developed internal lymphatic circulation (11). Results from previous studies show that drugs applied to the breast skin are selectively concentrated in the breast (12, 13), whereas drugs applied to the skin of other regions of the body penetrate the skin into the vascular system and are distributed systemically. Thus, transdermal drug application to the breast skin can be considered as local transdermal therapy (LTT), a concept which is further reviewed elsewhere (14). In previous studies, 4-hydroxytamoxifen (4-OHT) gel was applied to the breast skin in settings ranging from 2 to 3 weeks of preoperative treatment in postmenopausal women with invasive cancer to treatment of mastalgia in premenopausal women for up to 1 year (12, 13, 15). Because LTT is most suited to women with DCIS or those at high risk, we performed a presurgical randomized trial of LTT in women with DCIS, testing 4-OHT gel against oral-T. Here we report results from 26 evaluable subjects who completed the study before its closure because of expiration of the shelf-life of the 4-OHT gel, with no additional drug available.

Participants and Methods

Study design

Between November 2009 and March 2012, pre- and postmenopausal women (age range 45–86) with a diagnosis of ER-positive DCIS (as defined in ASCO/CAP guidelines; ref. 16) were recruited at Northwestern University and Washington University to a randomized, double-blind, placebo-controlled trial of LTT with 4-OHT gel versus oral-T during the window between diagnostic core needle biopsy and surgical excision (NCT00952731 or N01-CN-35157). Women at risk for thromboembolic disease were excluded, as were those with a history of exogenous hormone use within the past month, and tamoxifen or raloxifene use within the past 2 years. Randomization was stratified by menopausal status and enrollment site. Initially, the FDA required exclusions for grade 3 and comedo-type DCIS, mammographic DCIS size of >5 cm, and palpable lesions; these were removed following enrollment of 9 subjects in the first year.

Study medication

4-OHT gel (Besins Healthcare BHR Pharma, LLC) was formulated as 0.2% (w/v) gel containing 200 mg of 4-OHT (*E:Z* = 1:1) in 100 mL of hydroalcoholic, fast-drying gel supplied in a metered-dose container that dispensed 1.0 mL of gel (2 mg of 4-OHT or placebo) with each pump. Oral-T (20 mg) and placebo capsules were supplied by NCI, Division of Cancer Prevention: (Z)-tamoxifen tablets in opaque gelatin capsules filled with microcystalline cellulose powder.

Study procedures

All participants provided informed consent. Baseline assessments included a history and physical explanation of gel application, completion of the Breast Cancer Prevention Trial Eight Symptom Scale (BESS) questionnaire (17), collection of a venous blood sample, and collection of nipple aspirate fluid (NAF) from NAF yielders (18). Following randomization, study drug was shipped to participants. The gel group received 4-OHT gel (4 mg daily, 2 mg to each breast) and oral placebo; the oral group received tamoxifen capsules (20 mg daily) and placebo gel. Treatment began within 5 days after randomization and ended on the day before surgical resection. Participants were instructed to apply the gel to the entire skin envelope of each breast each morning, after a shower. Duration of therapy was 6 to 10 weeks. Compliance was assessed through participant diaries, counts of returned pills and of returned gel canisters. Participants who took at least 80% of the prescribed dose were considered compliant.

Assessments similar to those performed at baseline were repeated on the day before, or on the morning of surgery. During surgery, breast adipose tissue from the surgical sample was snap frozen and stored at −80°C for measurement of tamoxifen and metabolites. The samples were obtained from a location adjacent to the DCIS lesion to provide uniformity between participants undergoing breast conservation and mastectomy. The paraffin block of the core and excision samples were acquired by the recruiting institution and 10 sections from each specimen were submitted to the NU Pathology Core Facility. The sections were cut in batches (with pre- and posttreatment samples in the same batch), shipped cold, and processed for immunohistochemistry within 4 weeks.

The BESS Questionnaire was repeated at day 15 and at the end of treatment (1 day before surgery or day of surgery), and the postsurgical visit (approximately 7–14 days after

surgery). In an independent assessment at the same time points, adverse events were coded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

Study endpoints

The primary efficacy endpoint of this study was to demonstrate that daily application of 4-OHT gel to the breasts results in a reduction in the Ki67 labeling index (LI) of DCIS lesions, similar to that seen with oral-T, comparing the diagnostic core biopsy to the surgical excision sample. Secondary endpoints were (i) to compare concentrations of tamoxifen and its metabolites [4-OHT, endoxifen, N-desmethyl tamoxifen (NDT)] in breast tissue, plasma, and NAF obtained on the day of surgery; (ii) to assess changes in known tamoxifen-modulated pathways in the breast [cyclooxygenase-2 (COX2) and maspin protein expression; refs. 19 and 20], and plasma [sex hormone–binding globulin (SHBG), insulin-like growth factor-1 (IGFI); ref. 14]. Side-effect endpoints were (i) the incidence of hot flashes at baseline and before surgery; (ii) changes in coagulation-related proteins in women on the gel and the oral arms from baseline to immediately before surgery.

Ki67, COX2, and maspin expression

Immunohistochemical (IHC) assessment of these markers was performed on paraffin-embedded sections of the core and excision specimens, using standard IHC techniques and MCF7, HCT116, and H292 cells as controls. For maspin, we used primary mouse monoclonal antibody (Clone-G167-70; BD Pharmingen), dilution 1:200; for COX2, primary mouse monoclonal antibody (Clone-CX-294; Dako), dilution 1:100; and for Ki67, primary mouse monoclonal antibody (Clone-MIB-1; Dako), dilution 1:100 antigen. Dako Envision Plus system horseradish peroxidase–labeled polymer for 20 minutes at 37°C was used as the detection system. Scoring was performed on DCIS lesions only, with manual counting of positively stained DCIS cells. The Ki67 LI was assessed on an average of 300 DCIS cells at ×40 magnification. The *H*-score system (Score range: 0–300) was used for COX2 and maspin markers by a single observer who was blinded to treatment status, with random verification of 20% of slides by a pathologist (P. Kulesza; ref. 21).

Plasma and breast tissue concentration measurement of tamoxifen and its metabolites

(Z)-tamoxifen, (Z)-NDT, (E) and (Z)-4-OHT, and (Z)-endoxifen were measured by liquid chromatography/tandem mass spectrometry (LC/MS-MS) with a turbo ion spray interface operating in positive mode (API 3000; AB SCIEX). Briefly, 100 μL of plasma was mixed with 200 μL of acetonitrile containing 1 ng each of the deuterated analogs of the analytes (TRC), centrifuged at 4°C and 7,000 rpm for 10 minutes, and supernatant diluted with 200 μL of water before analysis. For analysis of NAF, samples were collected in a capillary tube and diluted with 200 μL of phosphate-buffered saline; 100 μL of the diluted NAF sample was used for analysis. Breast adipose tissue samples, 25 mg, were minced and treated with 125 μL of a 1 mg/mL arsenic solution in 2% nitric acid (Inorganic Ventures) and extracted as described above. Chromatographic separation was achieved with a Kinetex PFP 2.6μ column, 50 × 2.1 mm (Phenomenex). The mobile phase was A: 0.1% formic acid in water (v/v) and B: 0.1% formic acid in acetonitrile (v/v). The flow rate was 0.3 mL/min at 25°C. Retention times for (Z)-tamoxifen, (Z)-NDT, (Z)-4-OHT, (E)-4-OHT, and (Z)-endoxifen were 7.3, 6.8, 5.1, 4.7, and 4.5 minutes, respectively. Total run time was 13 minutes. Acquisition was performed in multiple reaction monitoring mode using m/z 372.2 → 72.1, 388.2 → 72.1, 374.2 → 72.1, and 358.2 → 72.1 at low resolution for tamoxifen, 4-OHT, endoxifen, and NDT, respectively. In 3 participants, matched samples were not available: breast adipose tissue was not collected in 2, and the plasma sample was missing in 1.

Because the fraction of E and Z isoforms of 4-OHT was of particular interest, we used an additional validated method to study plasma concentrations of these metabolites in a different laboratory (Eurofins Medinet). Plasma from blood samples collected in lithium-heparin tubes was frozen at −20°C, shipped in batches on dry ice to the Eurofins Medinet Central Laboratory; LC/MS-MS was used for the simultaneous determination of (E) 4-OHT and (Z) 4-OHT, with a lower limit of quantitation (LOQ) of 10 pg/mL and upper LOQ of 10,000 pg/mL. Eurofins Medinet developed and validated the method for BHR Pharma, in accordance with the FDA Guidance on Bioanalytical Method Validation (22).

Circulating marker assessment

Plasma samples collected with anticoagulant K_3-EDTA were used for human IGFI and SHBG assays and coagulation protein assays [factor VIII, factor IX, von Willebrand factor (vWF), and protein S; ref. 23]. The human IGFI and SHBG assays were performed with Quantikine Enzyme-Linked Immunosorbent Assay (ELISA) Kits (R&D Systems; cat. no. DG100 for IGFI and cat. no. DSHBG0 for SHBG assay). The lower limit of detection was 56 pg/mL and 5 pmol/L; %CV values were 4.3 and 5.6 for IGFI and SHBG assay, respectively. Factors VIII and IX were determined with VisuLize antigen ELISA Kits (Affinity Biologicals Inc.). vWF was measured with immune-turbidimetric assay (Diagnostica Stago Inc.; cat. no. 00518) by STA analyzer, and total protein S was assayed with an ELISA Kit (REAADS Inc.)

Statistical design and analysis

The study was powered to detect a 50% reduction in Ki67 LI from baseline to posttherapy, with the hypothesis that change would be similar in the 2 groups. Therefore, if the mean relative decrease in the 4-OHT group was at least 30%, this would be considered equivalent to a relative decrease of up to 50% in the tamoxifen group. With $\alpha = 5\%$ and $\beta = 20\%$, the planned sample size was 112 women, expecting that 90 would be evaluable for the primary endpoint of Ki67 LI. The study was halted early, but our assumptions about relative variability in the data and relative change from baseline have held for the main variable. In particular, the

baseline means for Ki67 are 8.3% and 6.7% in the oral and in the 4-OHT groups respectively, whereas the corresponding SDs are 5.2% and 5.6%. This gives the coefficients of variation of $5.2/8.3 = 0.63$, which is exactly what we assumed for the oral group and $5.6/6.7 = 0.84$, which is around 30% larger than what we assumed for the 4-OHT group.

For continuous variables in the immunochemistry, drug concentration and blood coagulation data, means, and standard deviations are reported; the significance of changes between baseline and posttreatment *within* groups were evaluated with the paired *t* test, and differences *between* treatment groups assessed using the unpaired *t* test. For categorized demographic data, we examined the association of these variables with treatment group via Fisher exact test. For the analysis of quality of life, the 33 symptoms in the BESS Questionnaire were divided into 8 clusters as described by Cella and colleagues (17). The mean score within each cluster was used to evaluate significance of changes from baseline to posttreatment within groups as well as the differences between treatment groups using the Wilcoxon signed-rank test.

Results

A total of 31 subjects were enrolled over 29 months (November 2009 to July 2011), at which point the shelf-life of the drug expired and the study was closed. Three participants were ineligible (2 with ER negative DCIS and 1 with high creatinine); one participant withdrew consent before randomization. Of 27 randomized participants, one was withdrawn from the study due to lack of drug supply. A total of 26 subjects completed the study, 14 in the oral-T group and 12 in the topical 4-OHT gel group (see Fig. 1). The range of therapy duration was 6 to 10 weeks and the median time on treatment was 6 weeks (see Table 1).

Baseline participant characteristics

Participant demographics and clinical characteristics according to treatment groups were not significantly different (see Table 1). In particular, there were no significant differences in DCIS grade, lesion size, ER and PR expression, age, or menopausal status.

Tissue markers. We were not able to obtain matched core and excision samples from 2 participants. Another 6 participants were excluded from analysis of IHC endpoints because the DCIS lesion had been exhausted in the baseline sample in 1, and there was insufficient DCIS remaining in the excision specimen in 5 additional participants. Thus, of 26 women completing the study, matched DCIS lesions from baseline and posttreatment specimens were not available on 8, yielding a total of 18 subjects who were evaluable for IHC markers (9 in the tamoxifen group and 9 in the 4-OHT gel group). The changes in Ki67 LI in DCIS lesions, according to the treatment group, are summarized in Table 2. The mean Ki67 LI after the treatment decreased

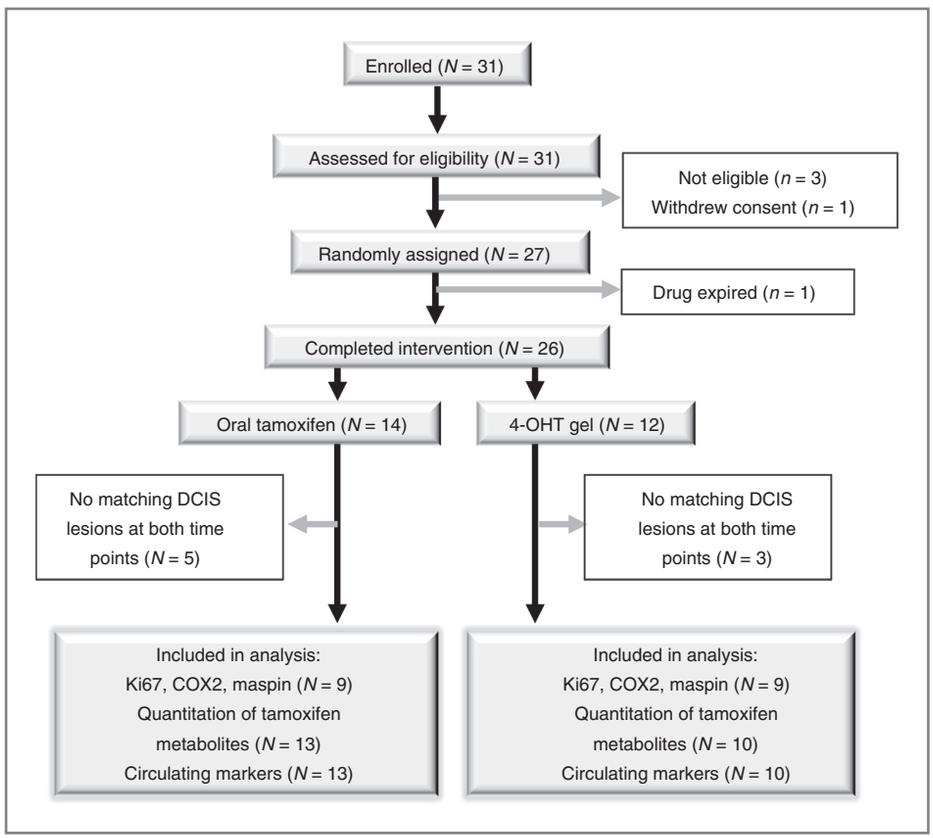

Figure 1. CONSORT diagram (participant flow diagram).

Table 1. Participant characteristics at baseline[a], DCIS size from surgical specimen, and the duration of treatment according to treatment groups

	Oral-T (20 mg/day)	4-OHT gel (4 mg/day)	*P*
No. of participants	$N = 14$	$N = 12$	
Age, y (IQR)[b]	54 (50, 61)	60 (52, 65)	0.29
Menopausal status			
Pre	3 (21.4%)	4 (33.3%)	0.67
Post	11(78.6%)	8 (66.7%)	
Race			
Caucasian	6 (42.9%)	7 (58.3%)	0.70
Non-Caucasian	8 (57.1%)	5 (41.7%)	
DCIS grade			
1	3 (21.4%)	1(8.3%)	0.69
2	10 (71.4%)	9 (75.0%)	
3	1 (7.1%)	2 (16.7%)	
DCIS size at surgery, cm (IQR)[b]	1.9 (0.6, 2.4)	0.58 (0.4, 1.36)	0.23
%ER expression (IQR)[b]	80% (67, 95)	85% (67, 100)	0.86
%PR expression (IQR)[b]	67% (26, 90)	67% (33, 75)	0.84
Days of treatment (IQR)[b]	44 (42, 47)	46 (45, 48)	0.29
40–59 days	12 (85.7%)	10 (83.3%)	
60–69 days	2 (14.3%)	2 (16.7%)	

[a]A total of 27 participants were randomized, but 26 participants completed the intervention.
[b]Values are reported in median with interquartile range (IQR).

significantly from baseline in both treatment groups (mean reduction 5.1% in tamoxifen group, $P = 0.008$ and 3.4% in the topical 4-OHT group, $P = 0.03$). This mean reduction in the 2 groups was statistically similar ($P = 0.99$).

COX2 and maspin changes by treatment are shown in Table 2. There were no significant differences between baseline and posttreatment in COX2 or maspin expression within each treatment group. Also, no differences were noted between treatment groups with respect to the baseline-to-posttreatment changes in these biomarkers ($P = 0.19$ for COX2; $P = 0.38$ for maspin).

Plasma and tissue concentrations of tamoxifen and its metabolites

There were a total of 23 participants (13 treated with tamoxifen and 10 with 4-OHT gel) whose plasma and breast

Table 2. Ki67, COX2, and maspin changes according to the treatment groups

	Oral-T (20 mg/day) ($N = 9$)		4-OHT gel (4 mg/day) ($N = 9$)		
	Mean ± SD	*P*[a]	Mean ± SD	*P*[a]	*P*[b]
Ki67					
Baseline	8.3 ± 5.2		6.7 ± 5.6		
Posttreatment	3.2 ± 2.3		3.2 ± 2.6		
Changes from baseline	−5.1 ± 5.5	0.008	−3.4 ± 5.0	0.03	0.99
COX2					
Baseline	67.2 ± 72.4		53.9 ± 55.7		
Posttreatment	78.9 ± 63.5		35.7 ± 28.9		
Changes from baseline	11.7 ± 109.8	0.46	−18.2 ± 32.2	0.44	0.19
Maspin					
Baseline	107 ± 56		120 ± 97		
Posttreatment	142 ± 69		162 ± 37		
Changes from baseline	35 ± 71	0.43	41 ± 115	0.23	0.38

Ki67 LI was represented in %; COX2 and maspin in *H*-score.
[a]Paired *t* test for changes from baseline within a treatment group.
[b]Unpaired *t* test for changes from baseline between treatment groups.

Table 3. Concentrations of tamoxifen and its metabolites in breast tissue (ng/g) and plasma (ng/mL)

	Breast adipose tissue			Plasma		
	Tamoxifen (20 mg/day)	4-OHT gel (4 mg/day)		Tamoxifen (20 mg/day)	4-OHT gel (4 mg/day)	
Analytes	($N = 13$)	($N = 10$)	P^a	($N = 13$)	($N = 10$)	P^a
(Z) Tamoxifen	2,959 ± 1,035	BQL		90 ± 45	BQL	
(Z) NDT	492 ± 192	BQL		149 ± 57	BQL	
(E) 4-OHT	BQL	5.2 ± 10.0		BQL	BQL	
(Z) 4-OHT	5.4 ± 2.8	5.8 ± 9.3	0.88	1.1 ± 0.7	0.2 ± 0.2	0.0003
(Z) Endoxifen	8.0 ± 6.8	BQL		5.9 ± 3.2	BQL	
Independent validation in Eurofins laboratory						
(E) 4-OHT				0.010 ± 0.006	0.056 ± 0.072	0.06
(Z) 4-OHT				1.488 ± 0.771	0.261 ± 0.284	<0.0001

NOTE: All the concentrations were reported as means ± SD. Tissue concentration was represented in ng/g (LLOQ = 3 ng/g). (E) isomers of NDT, and Endoxifen were BQL. Plasma concentration was represented in ng/mL (LLOQ = 20 ng/mL for tamoxifen and NDT; 1 ng/mL for 4-OHT and Endoxifen). (E) isomers of NDT, 4-OHT, and Endoxifen were BQL.

LLOQ of plasma concentration measured by Eurofins laboratory was 10 pg/mL for both E and Z isomers.

Abbreviations: BQL, below the lowest limit of quantification (LLOQ); Endoxifen, *N*-desmethyl 4-hydroxytamoxifen; 4-OHT, 4-hydroxytomoxifen.

[a]Unpaired *t* test between oral and topical treatment groups.

adipose tissue samples, matched pre- and posttreatment, were available for quantitation of tamoxifen and its metabolites (NDT, 4-OHT, and endoxifen). The mean plasma and tissue concentration of each analyte is reported in Table 3. Detectable levels of tamoxifen, NDT, and endoxifen were found only in the oral-T group, with mean values being substantially higher in tissue than in plasma. In contrast, (Z) 4-OHT was detectable in the tissue of both oral and gel groups, at equivalent concentrations (5.4 and 5.8 ng/g, respectively, $P = 0.88$), whereas plasma concentrations were markedly different (1.1 ng/mL in the oral-T group and 0.2 ng/mL in the 4-OHT gel group, $P = 0.0003$; see Table 3). (E) 4-OHT was present in breast adipose tissue of the gel group at a concentration of 5.2 ± 10.0 ng/g. Data on plasma (Z) 4-OHT concentrations from both laboratories were very similar, but the (E) 4-OHT assay was more sensitive in the Eurofins laboratory and (E) 4-OHT was detectable in plasma of both gel and oral groups, at levels that were considerably lower than those of (Z)4-OHT (see Table 3).

Overall, we found that 4-OHT was detectable in breast tissue for 9 of 10 participants in the 4-OHT gel group for whom samples were available; and in plasma for 5 participants. We observed a direct correlation between plasma and tissue concentration of (Z) 4-OHT in the oral-T group (Spearman correlation coefficient = 0.79, $P = 0.0007$); however, there was no such correlation in the 4-OHT gel group (Spearman correlation coefficient = 0.24, $P = 0.48$). We then looked at individuals with the highest (Z) 4-OHT tissue concentrations (≥1.5-fold of the mean). In the 4-OHT gel–treated group, there were 2 such participants, with 14.9 and 33.2 ng/g of (Z) 4-OHT in breast. However, their plasma (Z) 4-OHT was undetectable for one participant, and 0.22 ng/mL for the other, compared with the mean (0.2 ng/mL).

Drug concentrations in NAF

We obtained posttreatment NAF (3–40 μL) from the contralateral breast of 6 participants, and were able to detect tamoxifen or its metabolites in NAF samples of 4 participants, 2 in the tamoxifen and 2 in the 4-OHT gel group. In the oral-T group, NAF concentrations of tamoxifen and NDT were higher than the plasma concentrations (participant A: 320 ng/mL vs. 94.3 ng/mL for tamoxifen and 406 ng/mL vs. 133 ng/mL for NDT; participant B: 182 ng/mL vs. 160 ng/mL for tamoxifen and 355 ng/mL vs. 222 ng/mL for NDT) whereas 4-OHT and endoxifen were undetectable. In the 4-OHT gel group, tamoxifen, NDT, and endoxifen were undetectable, but both isomers of 4-OHT were detected in similar amounts, with (Z) 4-OHT concentration ≥40-fold higher than the plasma concentration (participant C: 16.7 ng/mL vs. 0.42 ng/mL; participant D: 25.1 ng/mL vs. BQL. Of the 2 NAF samples with undetectable tamoxifen or metabolites, 1 subject received oral-T, had detectable plasma tamoxifen (54 ng/mL) and NDT (136 ng/mL), but unfortunately no breast tissue sample was collected for drug quantitation at surgery. The other subject with undetectable tamoxifen or metabolites in NAF received active 4-OHT gel, and had a high breast adipose tissue 4-OHT level of 33.2 ng/g for (Z) 4-OHT.

Tamoxifen responsive–circulating markers

Mean baseline IGFI, SHBG, vWF, factor VIII, factor IX, and total protein S levels are shown in Table 4. Overall, these markers of systemic hormonal effects were induced in the tamoxifen group, but not in the 4-OHT group. Specifically, median SHBG levels increased significantly following tamoxifen therapy ($P = 0.002$), but not with 4-OHT gel therapy ($P = 0.67$). Mean vWF and factor VIII levels increased significantly with oral-T therapy ($P = 0.02$ and

Table 4. Changes in circulating markers according to the treatments

	Tamoxifen (20 mg/day) ($N = 13$)		4-OHT gel (4 mg/day) ($N = 10$)		
	Mean ± SD	P^a	Mean ± SD	P^a	P^b
IGFI (ng/mL)					
Baseline	59.0 ± 11.4		63.7 ± 8.6		
Posttreatment	50.3 ± 9.7		58.5 ± 6.6		
Changes from baseline	−8.7 ± 8.3	0.003	−5.2 ± 9.5	0.12	0.35
SHBG (ng/mL)					
Baseline	98.4 ± 45.0		89.4 ± 70.2		
Posttreatment	143.9 ± 69.0		99.7 ± 76.0		
Changes from baseline	45.5 ± 40.2	0.002	10.3 ± 74.4	0.67	0.20
%vWF					
Baseline	167.4 ± 89.2		179.9 ± 68.3		
Posttreatment	218.6 ± 134.6		177.3 ± 65.3		
Changes from baseline	51.2 ± 71.0	0.02	−2.6 ± 52.3	0.88	0.06
%Factor VIII					
Baseline	157.1 ± 47.5		158.4 ± 23.4		
Posttreatment	168.7 ± 51.6		167.1 ± 24.5		
Changes from baseline	11.6 ± 17.3	0.03	8.7 ± 18.5	0.17	0.70
%Factor IX					
Baseline	86.6 ± 8.8		86.7 ± 7.0		
Posttreatment	87.0 ± 12.2		81.1 ± 12.7		
Changes from baseline	0.4 ± 10.2	0.89	−5.6 ± 13.6	0.22	0.24
%Total Protein S					
Baseline	94.3 ± 8.9		97.5 ± 7.0		
Posttreatment	91.6 ± 12.2		95.9 ± 9.2		
Changes from baseline	−2.7 ± 9.3	0.32	−1.6 ± 6.5	0.46	0.76

[a]Paired *t* test between baseline and posttreatment value within a treatment group.
[b]Unpaired *t* test for changes from baseline between treatment groups.

0.03, respectively) but not with 4-OHT gel therapy ($P = 0.88$ and 0.17, respectively). The mean levels of factor IX and total protein S did not change for either treatment group. Finally, mean IGFI levels were significantly lower than baseline in the oral-T group ($P = 0.003$), but not in the 4-OHT gel group. However, between-group comparisons of these treatment-related changes did not reach statistical significance.

Quality of life assessment

Quality of life parameters assessed by BESS questionnaire are summarized in Table 5. At baseline, the mean scores for all clusters were similar for the 2 treatment groups, with the exception of the vaginal symptom cluster that was marginally higher in the 4-OHT gel group (0.14 compared with 0.00, $P = 0.052$). Following treatment, the mean score for vasomotor symptoms (hot flashes, night sweats, and cold sweats) increased slightly compared with baseline in both groups (oral-T group, $P = 0.06$ and 4-OHT gel group, $P = 0.13$), but these changes were not significantly different between the 2 treatment groups ($P = 0.83$). The gastrointestinal symptom cluster score was somewhat higher in the oral-T group at baseline, and although the within-group change was not significant in either oral or transdermal groups, the between group comparison did reach statistical significance ($P = 0.049$). There were no other between-group differences in the change of symptom severity from baseline to posttreatment. In addition, in our collection of CTCAE data, no serious adverse events were reported in this study.

Discussion

LTT to the breast for prevention of in-breast recurrence of DCIS and occurrence of new primary tumors is a promising approach with the potential of significantly reducing side effects through reduced systemic exposure. We report the first study of this approach in women with DCIS, comparing a proven breast cancer prevention agent (tamoxifen) given orally, and one of its active metabolites (4-hydroxytamoxifen) given transdermally to the breast for at least 6 weeks. Although we did not reach our target accrual, we report results on the crucial issue of drug concentration in blood and plasma, and preliminary data on biomarkers of efficacy (Ki67 labeling in DCIS tissue) and systemic exposure (plasma levels of IGFI, SHBG, and coagulation proteins).

Our primary endpoint was Ki-67 LI, which is the best validated and most widely accepted endpoint for window-of-opportunity studies of systemic agents for breast cancer

Table 5. Summary of BESS quality of life assessment by symptom clusters according to the treatments

	Tamoxifen (20 mg/day) ($N = 14$)		4-OHT gel (4 mg/day) ($N = 12$)		
Symptom cluster	**Mean ± SD**	**P^a**	**Mean ± SD**	**P^a**	**P^b**
Cognitive					
Baseline	0.62 ± 0.61		0.61 ± 1.11		0.54
Posttreatment	0.71 ± 1.18		0.69 ± 1.20		
Changes from baseline	0.10 ± 0.86	0.99	0.08 ± 0.47	0.81	0.64
Body pain					
Baseline	0.76 ± 1.12		0.56 ± 0.94		0.49
Posttreatment	1.19 ± 1.11		0.58 ± 0.74		
Changes from baseline	0.43 ± 0.92	0.11	0.03 ± 0.61	0.78	0.27
Vasomotor					
Baseline	0.33 ± 0.45		0.19 ± 0.41		0.35
Posttreatment	0.88 ± 1.26		0.53 ± 0.73		
Changes from baseline	0.55 ± 1.05	0.06	0.33 ± 0.64	0.13	0.83
Gastrointestinal					
Baseline	0.12 ± 0.31		0.00 ± 0.00		0.18
Posttreatment	0.02 ± 0.09		0.06 ± 0.13		
Changes from baseline	−0.10 ± 0.24	0.50	0.06 ± 0.13	0.50	0.049
Sexual problems					
Baseline	0.32 ± 0.72		0.42 ± 0.67		0.55
Posttreatment	0.11 ± 0.40		0.25 ± 0.5		
Changes from baseline	−0.21 ± 0.54	0.25	−0.17 ± 0.81	0.53	0.48
Bladder					
Baseline	0.14 ± 0.36		0.17 ± 0.39		0.87
Posttreatment	0.25 ± 0.33		0.25 ± 0.40		
Changes from baseline	0.11 ± 0.49	0.59	0.08 ± 0.19	0.50	0.67
Body image					
Baseline	0.68 ± 0.77		0.63 ± 0.83		0.55
Posttreatment	0.64 ± 0.89		0.75 ± 1.06		
Changes from baseline	−0.04 ± 0.87	0.92	0.13 ± 0.53	0.75	0.26
Vaginal					
Baseline	0.00 ± 0.00		0.14 ± 0.26		0.052
Posttreatment	0.07 ± 0.19		0.14 ± 0.33		
Changes from baseline	0.07 ± 0.19	0.50	0.00 ± 0.14	0.75	0.08

[a]Wilcoxon signed-rank tests were used for the changes from baseline within a treatment group.

[b]Wilcoxon rank-sum tests were used for baseline, and the changes from baseline between treatment groups.

(24). Encouragingly, although the power of our study to "prove" equivalence between groups is limited because only 28% of the subjects were accrued, our assumptions about variability in Ki67 LI and relative change from baseline have held, in that the baseline means for Ki67 LI in the 2 groups and the corresponding SDs and coefficients of variation are very close to the assumptions used for the statistical plan. The drop in Ki67 LI was larger than anticipated in both groups: 61% rather than 50% in the oral group and 52% rather than 30% in the 4-OHT group, consistent with the projected "effect size." Our findings are strengthened by an earlier study of postmenopausal women with ER-positive invasive cancers, where 2 to 3 weeks of treatment with up to 2 mg of 4-OHT gel (1 mg per breast) was compared to oral-T before cancer resection. Cell proliferation decreased to a similar degree with oral and transdermal therapy, and 4-OHT plasma concentrations were significantly lower in the transdermal group (13). In contrast, we included pre- and postmenopausal women, used a higher daily dose of 4-OHT (4 mg) and a longer treatment interval of 6 to 10 weeks to allow an assessment of vasomotor symptoms.

We assessed COX2 and maspin labeling of DCIS lesions because of previous evidence that their expression is modulated by tamoxifen (19, 25). Although significant modulation in COX-2 and maspin expression was not seen in either group, these potential markers of DCIS biology remain of interest (20, 26).

Our results support the hypothesis that effective breast concentrations can be achieved with low systemic exposure. The breast adipose tissue concentrations of (Z) 4-OHT were

equivalent in the oral and LTT groups (more than 5 ng/mg tissue in both groups). Our results compare favorably with the previous study where median 4-OHT concentration in nontumor breast tissue was 2.0 ng/g in the oral-T group and 0.8 ng/g in the 4-OHT gel group (2 mg daily; ref. 13). In contrast, the mean plasma level of 4-OHT was more than 5-fold lower in the 4-OHT gel group than in the tamoxifen group using 2 independent methods in different laboratories. Although data on NAF concentrations were available only on 4 women, these too support the main finding of high mammary concentrations of 4-OHT achieved with LTT, and are of interest because they imply a within-breast distribution of the drug that allows high concentrations to appear in nipple fluid. Thus, our pharmacokinetic results compare favorably with previous reports and suggest that LTT for breast cancer prevention and for DCIS therapy using 4-OHT gel should be effective. A reduction in long-term side effects remains to be demonstrated. Nevertheless, the data are encouraging and support the design of future studies.

We measured 4-OHT isomers because they differ in anti-estrogenic activity, with the (Z) isomer of 4-OHT and endoxifen being the major biologically active forms (27–29). The pure (Z) form is difficult to stabilize in the manufacturing process, and 4-OHT gel contains equal amounts of both isomers. We found that the concentrations of 4-OHT isomers were similar in breast adipose tissue in the oral and gel groups, whereas plasma concentrations were significantly lower in the gel group, with no evidence of isomerization (Z → E; Table 3). This contrasts with the result from an earlier topical study using ^{3}H labeled (Z) 4-OHT to the breasts (30), where the authors reported a progressive (Z → E) isomerization in breast tissue samples collected from 12 hours to day 7. Other topical 4-OHT gel studies reported total concentration of 4-OHT rather than concentration of each isomer (12, 13, 15). In agreement with previous studies (12, 13, 15), we did not see any further metabolic transformation of 4-OHT to endoxifen in the breast adipose tissue following topical 4-OHT gel administration.

Recently, endoxifen has attracted attention based on its greater abundance relative to 4-OHT in women on oral-T (31, 32), and a report that endoxifen causes proteosomic degradation of ERα and may have more selective anti-estrogenic effects (33). We found marginally higher concentrations of endoxifen than 4-OHT in the breast adipose tissue of the oral-T group, and it is possible that the combined presence of endoxifen and 4-OHT implies better efficacy. However, it remains reassuring that the magnitude of the posttherapy Ki67 decrease was similar in the oral and gel groups. Endoxifen and 4-OHT have equal binding affinity for the ER (34, 35) and *in vitro* transdermal permeability (36). Future studies using a gel formulation of endoxifen would therefore be of interest.

Tamoxifen has been reported to affect plasma levels of IGFI and SHBG (37–39), providing a measure of the pharmacologic action of tamoxifen upon the hormone axis. Previous reports document a decrease in plasma IGFI levels with tamoxifen therapy (37) and an increase in serum SHBG related to the estrogenic effect on the liver (38, 39), which is dose dependent (40). We observed significant decreases in IGFI and increases in SHBG in the tamoxifen group, but not in the 4-OHT gel group, supporting the notion that systemic effects of 4-OHT gel are small, if any. However, given the small sample sizes, the magnitude of the change was not statistically different between groups.

Similarly, the use of oral-T has been associated with changes in coagulation proteins such as vWF, factor VIII, factor IX, and total protein S (12, 40, 41). We found that the posttreatment levels of factor VIII and vWF were significantly increased posttherapy in the tamoxifen but not in the 4-OHT gel group. Thus, the avoidance of first-pass metabolism of tamoxifen in the liver potentially avoids changes in the clotting cascade that contribute to the prothrombotic effects of SERMS (41–43), a clear advantage.

Another significant adverse effect of SERM therapy relates to the induction of hot flashes in both pre- and postmenopausal women. The very low plasma concentrations observed following transdermal application of 4-OHT raises the possibility that hot flashes will be reduced by LTT with active tamoxifen metabolites (12, 15, 30). However, the lowest plasma level of tamoxifen metabolite exposure to cause hot flashes has not been defined, and it is possible that even low-level exposure may be sufficient to cause hot flashes. We did not observe a significant effect on hot flash frequency following a minimum of 6 weeks of therapy, although our data are clearly limited by small numbers.

Finally, population variation in the efficiency of tamoxifen metabolism, related to polymorphisms in the *CYP2D6* and other genes (44), may adversely affect efficacy of orally administered tamoxifen. LTT with an active tamoxifen metabolite circumvents the need for prodrug activation, potentially avoiding one source of low bioavailability of active (31, 32). Our finding supports this notion because topical gel application of 4-OHT achieved the similar breast concentration of 4-OHT compared with oral administration of tamoxifen.

An important question with transdermal delivery to the breast pertains to whether this is local therapy (higher concentrations in the breast than elsewhere) or systemic therapy (with similar concentrations throughout the body). Previous work has shown that 4-OHT applied to the breast skin results in 10-fold higher breast tumor levels than when it is applied to the arm or shoulder (12). Although this differential accumulation was attributed to the binding of 4-OHT to ERs present in the breast, receptor binding alone is insufficient to explain 4-OHT retention at the levels observed (45, 46). A more likely explanation relates to the embryologic origin of the breast as a skin appendage (i.e., a modified eccrine gland), so that the breast parenchyma and its skin envelope are a single unit with a well-developed internal lymphatic circulation (11), further evidenced by the fact that the skin and parenchyma of the breast drain to the same sentinel lymph nodes (47, 48). If breast retention of locally applied drugs is an anatomic rather than physiologic phenomenon, it predicts that other drugs applied to

the skin of the breast should also concentrate in the parenchyma to a greater degree than can be expected based on transdermal systemic delivery through the circulation. Thus, LTT may be applicable to a variety of agents as long as they are effective prevention agents and show sufficient dermal permeation.

Our trial was slow to accrue, particularly in the beginning. Few window-of-opportunity trials have been performed in women with DCIS, and despite the consensus among physicians that a surgical delay of 6 weeks was not risky, the majority of eligible subjects were unwilling to experience this delay. Furthermore, initially restrictive eligibility criteria, designed to minimize the likelihood that participants with a core biopsy showing DCIS had undiagnosed invasive disease, greatly decreased the pool of available subjects, and resulted in the recruitment of women with very small DCIS lesions, leading to an attrition of almost 30% in the assessment of biomarkers in matched pre- and posttherapy lesions. Ultimately, slow accrual led to expiration of the study agent, and a decision by the manufacturer to not produce additional supplies. In future studies, it is clear that enrollment criteria should be as open as possible.

In summary, our data support the notion that local transdermal drug delivery to the breast will achieve sufficient drug concentrations to be effective, with low systemic exposure. This concept deserves further testing with 4-OHT, and is likely to be applicable to other lipophilic drugs with low molecular weight.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The authors take full responsibility for the design of the study, the collection of the data, the analysis and interpretation of the data, the decision to submit the article for publication, and the writing of the article.

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