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# Lack of Attenuation in the Antitumor Effect of Tamoxifen by Chronic CYP Isoform Inhibition

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*Tamoxifen is a selective estrogen receptor modulator used in estrogen receptor-positive breast cancer. Tamoxifen is metabolized to an extremely potent antiestrogen by cytochrome P450 (CYP) 2D6, 2C9, and 3A isoforms. The selective serotonin reuptake inhibitors (SSRIs) are potent inhibitors of these CYPs. Since the prevalence of depression in breast cancer patients is nearly triple that of the general population, it is likely that a subgroup of breast cancer patients will receive long-term treatment with both an SSRI and tamoxifen. A case control design was used to investigate the possibility that a resultant decrease in production of the 4-hydroxy metabolite from chronic inhibition results in the attenuation of the antitumor effect of tamoxifen. Twenty-eight patients without recurrences of breast cancer (controls) were matched to an equal number of cases (recurrences) by cancer stage and year of diagnosis. Data were analyzed on all chronic medication exposure (> 3 months) in both cases and controls classified as*

*to their status as CYP 2D6, 2C9, and 3A inhibitors, substrates, or inducers. No significant difference was found for CYP inhibitor or substrate exposure between cases and controls. Indeed, controls showed a slightly greater exposure to inhibitors of the relevant CYP isoforms compared to cases. These results suggested a trend toward the null hypothesis. It is unlikely that the effect of chronic exposure to potent CYP isoform inhibitors affects the antitumor effect of tamoxifen and its 4-hydroxy metabolite, supporting the safety of the continued practice of concomitant SSRI administration to breast cancer patients with depression.*

**Keywords:** Selective serotonin reuptake inhibitors; tamoxifen; estrogen receptor-positive breast cancer; depression; CYP isoform inhibition

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**T**amoxifen is a selective estrogen receptor modulator (SERM) used in both pre- and postmenopausal women with estrogen receptor-positive breast cancer.<sup>1,2</sup> Tamoxifen is oxidized to three metabolites: 4-hydroxy-tamoxifen, N-desmethyl-tamoxifen, and trans-tamoxifen. Although only 2% of tamoxifen is converted to 4-hydroxy-tamoxifen, it likely contributes the majority of the antitumor response since it is at least 100 times more potent as an antiestrogen than either the parent compound or the other two metabolites.<sup>3</sup> The 4-hydroxy metabolite is generated exclusively by the action of three isoforms of cytochrome P450 (CYP) on tamoxifen—2D6, 2C9, and 3A—with a pronounced intersubject variability in the relative percent contribution of each isoform.<sup>4</sup> CYP 2D6, 2C9, and 3A are all

susceptible to inhibition from a broad array of compounds.<sup>5</sup>

The selective serotonin reuptake inhibitors (SSRIs) are among the most potent inhibitors of one or more of the CYP isoforms involved in tamoxifen metabolism and have been in widespread use for more than 10 years. The 20% to 30% prevalence of depression in breast cancer patients is approximately two to three times greater than the general population.<sup>6-8</sup> Since the SSRIs are the most commonly used drugs for depression, it is likely that a significant subgroup of breast cancer patients will receive long-term treatment with both an SSRI and tamoxifen. Hence, the possibility exists that the production of the potent antiestrogenic 4-hydroxy metabolite may be chronically inhibited, resulting in the attenuation of the antitumor effect of tamoxifen.

Because of these issues, the aim of this study was to investigate whether chronic administration of CYP isoform inhibitors with tamoxifen is associated with an alteration in the clinical outcome of patients with estrogen receptor-positive breast cancer.

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## METHODS

We conducted a case control study to determine if the use of CYP isoform inhibitors by women taking tamoxifen was associated with a poorer outcome.

### Case-Patient Identification and Eligibility

The Regional Oncology Center at the State University of New York Upstate Medical University Hospital is a principal referral site for oncology care in central New York. Information concerning tumor status on individual patients from 1986 to present is retrievable from an electronic database in the tumor registry. Updates regarding the status of tumor recurrence for individual patients are requested from referring physicians annually.

The cohort containing the pool of potential cases in this database comprised patients on tamoxifen, initially diagnosed between 1986 to 1999 (inclusive), who had recurrences of estrogen receptor-positive breast cancer. Further eligibility for inclusion of cases was contingent on the availability of continuous annual updates and the presence of a complete medical record from the date of original tumor diagnosis until patient death or year 2003. Eligibility from this pool of 105 cases was further limited to patients with Stage II and III disease at year of diagnosis. This population was chosen to better maximize study power because Stage II/III patients account for the preponderance of relapses.<sup>1,2</sup> To militate against bias toward the null hypothesis, charts of potential cases were reviewed and patients excluded for disease contraindications and drug-drug interactions that preclude the use of chronically administered, potent CYP 2D6, 2C9, or 3A inhibitors. These factors included liver disease, long QT and neuroleptic malignant syndromes, and use of monoamine oxidase inhibitors.<sup>5</sup> At the end of this process, 28 patients were eligible for inclusion as cases.

### Controls: Source and Matching

Controls were selected from the same cohort as the cases. Identical eligibility criteria for cases (excluding tumor recurrence) were applied to controls. That is, controls were selected from the pool of estrogen receptor-positive patients initially diagnosed between 1986 and 1999 (inclusive), without recurrences, who had annual tumor status updates and longitudinal medical records available. The same drug-related exclusions for the cases also applied to the controls. To assist with matching by year of diagnosis, the inclusion period for initial tumor diagnosis of 1986 to 1999 was divided

into quartiles. Controls were then matched to cases by cancer stage at diagnosis and year of diagnosis. For purposes of study efficiency, 28 cases were matched to an equal number of controls for the initial (1:1) analysis to determine a trend toward statistical significance (greater inhibitor exposure in cases). An expansion of the study to a ratio of 1:4 was planned if we found a trend toward greater CYP inhibitor use in cases.

### Data Collected

Clinical features of all patients were extracted from the charts, stored on site at University Hospital. These records include all outpatient and inpatient progress notes, orders, summaries, telephone communications, and all laboratory and radiographic reports. Variables were entered on a predesigned data form. Age, sex, ethnicity, alcohol use, cigarette use, drug allergies, and all medication history lists available at each visit were recorded. No topical dermatological preparations were recorded in any patient. Drugs prescribed for less than 1 month (e.g., antimicrobials) and those discontinued with less than a 3-month exposure were excluded from data collection and analysis since it is doubtful that such short-term modulation of tamoxifen metabolism could significantly affect an ultimate clinical outcome. Since no patient in either group was chronically exposed to an inducer, all other medications prescribed were then reviewed to determine whether they were CYP 2D6, 2C9, or 3A4 inhibitors or substrates, as described in Table I.<sup>5</sup> Furthermore, as all of these medications are usually prescribed for indefinite periods, it was assumed that patients had continuous exposures unless discontinued in the medical record in both cases and controls.

The date for all data extracted was limited to year of collection to comply with the Health Insurance Portability and Accountability Act (HIPAA) guidelines for studies that do not require patient informed consent. The study was approved by the Institutional Review Board for the Protection of Human Subjects at the SUNY Upstate Medical University.

### Statistical Analysis

The main focus of the statistical analysis was the comparative exposure of CYP 2D6, 2C9, and 3A inhibitors between cases and controls. Although the pharmacokinetic impact from concomitant CYP 2D6, 2C9, and 3A substrate use with tamoxifen is much more speculative, comparative exposures to relevant CYP substrates were also separately analyzed for the sake of completeness. Given the significant intersubject variability in

**Table I** Classification of Chronic Medication Exposure in Population Receiving Tamoxifen

	CYP 2D6			CYP 2C9			CYP 3A		
	Inducer	Inhibitor	Substrate	Inducer	Inhibitor	Substrate	Inducer	Inhibitor	Substrate
Alprazolam									x
Amitriptyline			x			x			
Amlodipine									x
Atorvastatin									x
Codeine			x						
Diazepam									x
Diltiazem								x	x
Felodipine									x
Fluoxetine		x	x						
Ibuprofen						x			
Imipramine			x						
Losartan						x			
Lovastatin					x				x
Metoprolol			x						
Naproxen						x			
Nefazodone								x	
Nifedipine									
Paroxetine		x	x		x				
Progesterone									x
Propranolol			x						x
Quinine									x
Sertraline		x			x				
Simvastatin									x
Terfenadine									
Venlafaxine			x						
Verapamil									x
Warfarin						x			
Zolpidem									x

the relative contribution of each isoform to 4-hydroxy-tamoxifen generation and the variability in the  $K_i$  values among CYP inhibitors, exposures to all inhibitors and substrates were treated as discrete (yes/no) variables without weighting as to the degree of CYP isoform effect. Individual inhibitors or substrates of more than one of the isoforms involved in tamoxifen metabolism were counted more than once for analysis as a separate entity. Likewise, combinations of CYP2D6, 2C9, and 3A inhibitors or substrates used in individual patients were analyzed separately.

Patient characteristics between cases and controls were compared using the Yates-corrected chi-square test for categorical data and the Wilcoxon rank sum test for continuous data. When expected cell counts were less than 5, Fischer's exact test was used to determine differences between the groups using the EPI 6 statistical program (USD, Inc., Stone Mountain, GA). Matched

pair analysis (the maximum likelihood estimate) was used to assess the association between CYP inhibitor exposure and clinical outcome to strengthen the statistics.<sup>9</sup> The matched analysis was performed using the matched-samples component of the Pepi computer programs for epidemiologists (Version 4.0, Abramson JH, Gahlinger PM). This approach was repeated to compare CYP 2D6, 2C9, and 3A substrate exposure between cases and controls.

## RESULTS

Demographics for the two groups are shown in Table II. There were no statistically significant differences in patient characteristics between cases and controls. Specifically, no significant differences in ethnicity between cases and controls were observed since the overwhelming majority of patients was Caucasian. Table II

**Table II** Patient Characteristics

Variable	No		p-Value
	Recurrence (n = 28)	Recurrence (n = 28)	
Sex (%)			
Female	100	100	
Mean age at diagnosis (years)	57.53	51.29	0.11
Race (n)			
Caucasian	26	28	
African American	1	0	
Asian Indian	1	0	
Stage of breast cancer (n)			
II	24	24	
Node (-)	5	10	0.22
Node(s) 1-3	8	7	
Node(s) 4-9	7	2	0.14
Nodes > 9	1	2	
Not recorded	3	3	
III	4	4	
Node (-)	1	0	
Node(s) 1-3	0	0	
Node(s) 4-9	1	4	0.35
Nodes > 9	2	0	
Smoker (n)	7	5	
Alcohol users (n)	3	3	0.51
Allergy to 2D6, 2C9, or 3A (n)			
Inhibitor	2	1	
Substrate	1	2	
Year at diagnosis (n)			
1986-1993	4	4	
1994-1996	12	12	
1997-1999	12	12	

Node status refers to the number of nodes that showed tumor at the time of original breast cancer diagnosis. 2D6, 2C9, and 3A refer to individual isoforms of cytochrome P450 (CYP).

also shows data collected for the confounders of disease severity (age and smoking). Although there was a trend for the cases to be older and to have a greater smoking exposure than controls, neither factor reached statistical significance. There were an equal number of cases and controls exposed to alcohol, which, like cigarette smoke, is a potential confounder, possibly affecting the rate of drug metabolism.<sup>5</sup> The two groups had identical patient numbers with listed allergies to CYP 2D6, 2C9, and 3A inhibitors and substrates.

Table II also shows that cases and controls were identically matched for cancer stage and quartile year of initial diagnosis. However, within Stage II, more controls (10 vs. 5) were node negative, and more cases (7 vs. 2) had four to nine positive nodes. Since both ob-

**Table III** Matched Analysis of CYP Inhibitor and Substrate Exposure

	Case Exposed (n)	Control Exposed (n)	Odds Ratio	95% Confidence Interval
Inhibitor				
2D6	6	8	0.75	0.26-2.16
2C9	4	6	0.67	0.19-2.36
3A	1	1	1.00	0.06-15.99
2D6 + 2C9	4	6	0.67	0.19-2.36
Any inhibitor	6	8	0.75	0.26-2.16
Substrates				
2D6	9	2	4.5	0.97-20.83
2C9	4	1	4.0	0.45-35.79
3A	4	1	4.0	0.45-35.79
2D6 + 3A	6	4	1.5	0.42-5.32
Any substrate	10	5	2.0	0.68-5.85

servations could insert bias in favor of the alternative hypothesis, concomitant use of CYP 2D6, 2C9, and 3A inhibitors was evaluated in these 24 patients. Within these nodal subsets, 3 cases and 4 controls were exposed to relevant CYP inhibitors, favoring the null hypothesis. For Stage III, since more controls (4 vs. 1) had four to nine positive nodes, any resultant bias would favor the null hypothesis.

Table III shows the results of separate matched analyses for exposures to the relevant CYP inhibitors and substrates between cases and controls. No significant differences for either category were found. Indeed, for the primary exposure of interest (concomitant CYP inhibitor use), controls actually received a greater number of CYP 2D6 or 2C9 inhibitors, combined 2D6/2C9 inhibitors, and any inhibitor of the three isoforms. This observation of a trend toward protection from combined CYP inhibitor and tamoxifen exposure in the controls favors the null hypothesis since this concomitant use would likely produce a chronic decrease in the potent antiestrogen metabolite in the group with a better clinical outcome.

Table III also shows no significant differences between cases and controls concerning CYP 2D6, 2C9, and 3A substrate exposure. Although a trend toward a greater exposure to concomitant CYP isoform substrates in cases was observed, the significance of this observation is unclear since there is a lack of evidence supporting either the in vitro or the clinical relevance of drug interactions involving substrate use with tamoxifen.

## DISCUSSION

We found no significant impact on the clinical outcome from chronic exposure to CYP isoform inhibitors of tamoxifen oxidation in a population of estrogen receptor-positive breast cancer patients receiving concomitant tamoxifen treatment. We chose a case control study design due to the pilot nature of the hypothesis, the statistical power inherent in this design to determine a significant trend (if any), and the ability of this design to uncover the effect of an exposure on a long-latent clinical outcome.<sup>10</sup> These considerations in design, combined with the results of a matched analysis for relevant CYP inhibitor exposure, maximized our ability to uncover a trend toward the alternative hypothesis if one were present.

Several weaknesses are present in our study that could contribute to the negative results. The case control design is retrospective and particularly prone to bias.<sup>9</sup> Indeed, due to the inexactitude of the literature on the relative contributions of the three CYP isoforms to the production of the most potent active metabolite, 4-hydroxy-tamoxifen, incorrect assumptions could have been made that could affect our analysis and conclusions. Also, a substantial and unverifiable assumption was made as to the actual duration of exposure to all coadministered drugs, thereby producing a widely variable exposure to concomitant medications throughout the study period. However, any bias entered by either of these considerations would apply to both cases and controls without preference for either subgroup, particularly since patients in both groups were derived from a cohort nested in a database that is used for purposes other than research (clinical and administrative).

Since a beta error introduced by a small sample size could also account for the lack of an association between CYP inhibitor exposure and breast cancer recurrence, we applied statistics to address this question of adequate study power. Setting the power value at 90%, an alpha of .05, an odds ratio of 1.5, and a presumed inhibitor use in cases of 30% (based on the prevalence of depression), 719 cases would be required to detect a difference if one truly existed.

Our findings strengthen the *ex vivo* observation that 4-hydroxy-tamoxifen accumulates in tumors extracted from estrogen receptor-positive breast cancer patients with acquired tamoxifen resistance.<sup>11</sup> This observation, combined with our results, minimizes the likeli-

hood that pharmacokinetic alterations in tamoxifen metabolism are of major significance in permitting tumor recurrences in breast cancer patients. It is more likely that a pharmacodynamic shift to the right in dose response, perhaps via an alteration in estrogen receptor sensitivity within tumor cells to 4-hydroxy-tamoxifen, may occur in a subgroup of patients with estrogen receptor-positive breast cancer.

In summary, it is unlikely that the effect of chronic exposure to potent CYP isoform inhibitors affects the antitumor effect of tamoxifen and its 4-hydroxy metabolite. These observations support the safety of the continued practice of treating depression with SSRIs in breast cancer patients receiving concomitant tamoxifen therapy.

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