RESEARCH ARTICLE

Effects of SULT1A1 Copy Number Variation on Estrogen Concentration and Tamoxifen-Associated Adverse Drug Reactions in Premenopausal Thai Breast Cancer Patients: A Preliminary Study

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Abstract

Tamoxifen is a pharmacological estrogen inhibitor that binds to the estrogen receptor (ER) in breast cells. However, it shows an estrogenic effect in other organs, which causes adverse drug reactions (ADRs). The sulfotransferase 1A1 (SULT1A1) enzyme encoded by the SULT1A1 gene is involved in estrogen metabolism. Previous research has suggested that the SULT1A1 copy number is linked with the plasma estradiol (E2) concentration. Here, a total of 34 premenopausal breast cancer patients, selected from the Thai Tamoxifen (TTAM) Project, were screened for their SULT1A1 copy number, plasma E2 concentration and ADRs. The mean age was 44.3 ± 11.1 years, and they were subtyped as ER+/ progesterone receptor (PR)+ (28 patients), ER+/ PR- (5 patients) and ER-/PR- (1 patient). Three patients reported ADRs, which were irregular menstruation (2 patients) and vaginal discharge (1 patient). Most (33) patients had two SULT1A1 copies, with one patient having three copies. The median plasma E2 concentration was 1,575.6 (IQR 865.4) pg/ml. Patients with ADRs had significantly higher plasma E2 concentrations than those patients without ADRs (p = 0.014). The plasma E2 concentration was numerically higher in the patient with three SULT1A1 copies, but this lacked statistical significance.

Keywords: SULT1A1 - copy number variations - adverse drug reaction - estrogen - tamoxifen - breast cancer

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Introduction

Tamoxifen (TAM) has been widely used for anti-hormone therapy and provides a pharmacological effect in estrogen receptor (ER) positive (ER+) breast cancer patients, decreasing the recurrence of breast cancer and mortality rate by 50% and 30%, respectively (Klein et al., 2013). TAM is the trans-isomer of a triphenylethylene derivative (Dhingra, 1999) and has been used as an ER antagonist, where it competes with estrogen for binding to the ER and prevents cancer cell growth by its antiproliferative effect (Dhingra, 1999; Klein et al., 2013). In the absence of TAM treatment, estradiol (E2) exerts its activity by binding to the ERs present in the mammary gland and inducing a conformational change that then allows the link to co-activators to express cell proliferation (Del Re et al., 2012; Yang et al., 2013). In contrast, the binding of TAM to the ER increases the association with co-repressors that then actively inhibits gene transcription (Del Re et al., 2012). Furthermore, the ratio of the co-activators to co-repressors available to interact with the TAM-bound ERs determines whether TAM will behave as an antagonist or an agonist in different target organs (Dhingra, 1999). For example, TAM acts as ER-antagonist in the mammary gland but as an ER-agonist in the uterus where it can subsequently cause endometrial thickness (Dieudonne et al., 2014) or endometrial carcinoma (Maximov, 2013), a serious adverse drug reaction (ADR). However, the most common TAM-associated ADR is hot flashes (Kiyotani et al., 2012; Lorizio et al., 2012; Westbrook and Stearns, 2013), which result from the cessation of bound E2 on the ER.

Estradiol is mainly produced in the ovary and shows marked changes in plasma levels during the menstrual cycle in premenopausal women, while plasma E2 levels are quite stable during a given month in postmenopausal women, which reflects the different E2 production sources. In postmenopausal women the plasma E2 is derived from transformed estrone (E1), which accounts for its low plasma concentration compared with the plasma E2 concentrations in premenopausal women (Gjerde et al., 2008). For example, premenopausal women have

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been reported to have a significantly higher ($p < 0.001$) plasma E2 concentration (median 96.5 pg/ml; range: 69.0-154.0 pg/ml) than postmenopausal women (median 12.5 pg/ml; range 12.5-18.0 pg/ml) (Matsui et al., 2013). For breast cancer patients, the plasma E2 concentrations were reported to range widely from 10.0-1,150.0pg/ml in premenopausal breast cancer patients, depending on their menstrual patterns (Berliere et al., 2013), while in postmenopausal breast cancer patients they were less varied and lower at a median of 2.6 pg/ml and range of 0.4-14.9 pg/ml (Gjerde et al., 2010).

For both premenopausal and postmenopausal breast cancer patients, TAM is an estrogen antagonist of choice. However, previous studies have focused on the plasma E2 concentration in postmenopausal patients due to its stable concentration during the month (Gjerde et al., 2008) compared with the E2 concentrations in premenopausal women. Even though plasma E2 concentrations are difficult to compare among premenopausal patients due to the high degree of variability within the menstrual cycle, the study of plasma E2 concentrations and its metabolic pathway should not be neglected due to the outstanding pharmacological effect of TAM as an anti-estrogen agent in premenopausal breast cancer treatment.

Estrogen metabolism also involves phase I (cytochrome P450) and phase II (sulfotransferase) metabolic enzymes, as well as the 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD 1) and type 2 (17β-HSD 2) that reversibly convert E1 and E2 (Gjerde et al., 2010; Kallstrom et al., 2010). It was previously reported that *CYP2D6, CYP3A5* and SULT1A1 polymorphisms did not affect the plasma E1 and E2 concentrations, whereas the SULT1A1 copy number was positively related to the plasma E1 and E2 concentrations ($p=0.024$ and 0.010, respectively) (Gjerde et al., 2010). The plasma E2 concentration was significantly decreased (level change -18.2%; $p=0.004$), while the E1 concentration was numerically but not significantly decreased (level change -6.9%; $p=0.093$), after TAM was administered in postmenopausal breast cancer patients (Lonning et al., 1995). In contrast, the plasma E2 concentrations were reported in a separate study to be significantly increased by 239% from 28 to 95pg/ml ($p < 0.05$) and the E1 concentrations to be numerically but not significantly increased by 264% from 42 pg/ml to 153 pg/ml ($p=0.06$) in postmenopausal patients, while the plasma E1 and E2 concentrations were numerically but not significantly increased in premenopausal patients (Lum et al., 1997). Previous studies have suggested that the presence of two copies of the SULT1A1 gene (SULT1A1x2) was the most common genotype in the Caucasian (65.6-67.5%) (Gjerde et al., 2010; Moyer et al., 2011) and Japanese (65.0%) (Yu et al., 2013) populations, while the copy number variation (CNV) ranged from one to five copies (Gjerde et al., 2010; Moyer et al., 2011; Yu et al., 2013). The SULT1A1 copy number has been suggested to be strongly associated with the SULT1A1 enzymatic activity ($p=0.008$), where patients with higher copy numbers of SULT1A1 genes showed a higher enzyme activity than those patients with just two copies (Yu et al., 2013).

However, the relationship between the SULT1A1 CNV and the plasma E2 concentration has never been reported in Thai breast cancer patients. The objective of the present study was to determine the prevalence of SULT1A1 CNV, the plasma E2 concentration and TAM-associated ADRs, as well as their association in premenopausal Thai breast cancer patients undergoing TAM treatment.

Materials and Methods

Patients and sample preparation:

Premenopausal breast cancer patients were randomly selected from the Thai Tamoxifen (TTAM) Project, for those patients who were taking 20 mg TAM once daily and visited the oncology outpatient clinic at King Chulalongkorn memorial hospital between February and March 2015. All patients were older than 18 years with normal hepatic and renal functions (aspartate aminotransferase and alanine aminotransferase <2 upper normal limit, serum creatinine <1.2 mg/dL) in the four weeks prior to selection for this study. Patients must have filled a prescription for TAM for at least 2 months and their medication adherence was evaluated by self-report. Their medication record was screened for drug-drug interaction by a clinical pharmacist. Patients who reported less than 80% adherence to the TAM administration regime, showed evident drug-drug interaction or were diagnosed for psychiatric illness/cognitive impairment were excluded from the study. For the remaining 34 patients, who then formed the study group, 10 ml of whole blood was drawn from each patient by a professional nurse and stored in a Vacutainer® $_{K2EDTA}$ tube (BD, USA). The whole blood was separated into buffy coat and plasma sections. DNA extraction was prepared from the buffy coat section by using QIAamp® DNA Mini kit (Qiagen®, Netherlands) and used to determine the SULT1A1 copy number, while the plasma fraction was used for the E2 quantification. All DNA and plasma samples were stored at -20°C and -80°C, respectively, until analysis.

The study protocol was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (IRB No.406/57).

Determination of the SULT1A1 *copy number:*

The DNA samples were diluted to a final concentration of 10 ng/μl with autoclaved ultra-pure water. The analysis was performed by TaqMan® Universal PCR Master Mix, TaqMan® Drug Metabolism Copy Number Assay Sets, Reference Assay for Copy Number determination with a Step One Plus real-time® PCR system (Applied Biosystem, USA) and ViiA7 software (Applied Biosystems, USA). The thermal cycling for the qPCR in the TaqMan assay was 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

Estradiol (E2) quantification:

The Estradiol (E2) Bioassay™ ELISA Kit (Human) (United States Biological, USA) was employed to perform the plasma E2 concentration quantification, with all samples being analyzed within 6 months after sample collection. The detection range was 250-5,000 pg/ml and the sensitivity of the test was 1 pg/ml. The color intensity

was inversely proportional to the E2 concentration in the plasma sample and was measured spectrophotometrically at 450 nm in multi-label counter (VICTOR3; PerkinElmer, USA). The plasma samples were brought to room temperature and mixed gently prior to the assay. Due to the low expected and found plasma E2 concentrations in the premenopausal breast cancer patients, sample dilution was not necessary in this study and blank plasma was subsequently used instead of PBS (dilution buffer). All standards (100 ul/well) and plasma samples (100 ul/well) were added in duplicate to the wells of a 96-well plate. Standard E2 concentrations of 0, 250, 500, 1,000, 2,500 and 5,000 pg/ml were prepared for the calibration curve (E2 concentration versus the optical density) and the best fit line was derived by regression analysis. The E2 concentration of each sample was calculated by interpolating from the constructed E2 calibration curve.

TAM-associated ADRs

TAM-associated ADRs were individually evaluated by the hospital pharmacist based on data recorded in the patient's medical profile and from face-to-face interviews with the patients at recruitment time.

Data analysis

Data analysis was conducted by descriptive statistics using the SPSS (version 22) software. The Kolmogorov-Smirnov and Shapiro-Wilk tests were performed for normality tests, while the Kruskal-Wallis and Mann-Whitney U tests were performed for hypotheses testing of the non-parametric data.

Results

The 34 premenopausal breast cancer patients were categorized as being in stages 0-III of breast cancer (1, 13, 14 and 6 patients for stages 0 to III, respectively), with a mean age of 44.3±11.1 years and BMI of 23.4±3.6. The average duration of TAM administration in their treatment was 15.9 (IQR 34.1) months. Their hormone receptor statuses were ER+/PR+ (28 patients), ER+/PR- (5 patients) and ER-/PR- (1 patient). Three patients (8.8%) reported having TAM-associated ADRs, which were irregular menstruation (2 patients) and vaginal discharge (1 patient) (Table 1). Most (33/34; 97.1%) patients had two copies of the SULT1A1 genes, while only one patient (2.9%) had three copies. The median plasma E2 concentration was 1,575.6 (IQR 865.4)pg/ml and ranged from 415.0-4,186.5 pg/ml. The regression equation of the E2 calibration curve was $y=-8.76 \times 10^{-5}(x) + 0.43$ (R^2=0.864). Those three premenopausal patients who reported their TAM-associated ADRs had significantly higher E2 concentrations than those patients without reported ADRs (p=0.014, Table 2, Figure 1) but there was no significant (p=0.065) difference in the E2 concentrations among the two different TAM-associated ADRs symptoms (Table 2). The E2 concentration showed a trend to increase with more SULT1A1 copy numbers but, subject to the low sample size, this was not statistically

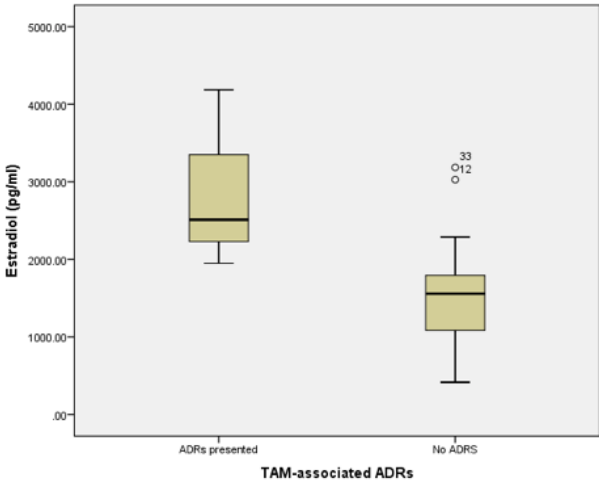

Figure 1. Estradiol (E2) concentration (pg/ml) between different TAM-associated ADRs. (The E2 concentrations of patient number 12 and 33 were 1,557.6 and 1,583.2 pg/ml, respectively.)

Table 1. Demographic and clinical data of the premenopausal thai breast cancer patients

Demographic and clinical data: Frequency (%) (N = 34)			
Age (years)	**No. (%)**	**TAM administration**	**No. (%)**
18-30	1 (2.9)	< 6 months	6 (17.6)
30-40	13 (38.2)	6 months-1 year	5 (14.7)
40-50	17 (50.0)	1 year-3 years	12 (35.3)
> 50	3 (8.8)	> 3 years	11 (32.4)
Hormone receptor status	**No. (%)**	**TAM-associated ADRs**	**No. (%)**
ER+	33 (97.0)	Irregular menstruation	2 (5.9)
PR+	28 (82.4)	Vaginal discharge	1 (2.9)

Table 2. Estradiol (E2) concentrations in different SULT1A1 copy numbers and TAM-associated ADRs

***SULT1A1* copy number**	**N**	**E2 concentration (pg/ml)**	**p-value**
SULT1A1 *x 2 copies*	33	1,567.9 (IQR 829.1)	0.353[a]
SULT1A1 *x 3 copies*	1	2,280.8 (IQR 0.0)	
TAM-associated ADRs	**N**	**E2 concentration (pg/ml)**	**p-value**
No ADRs presented	31	1,557.6 (IQR 740.9)	0.014*[a]
ADRs presented	3	2,511.2 (IQR 0.0)	0.065[b]
Irregular menstruation	2	3,067.5 (IQR 0.0)	
Vaginal discharge	1	2,511.2 (IQR 0.0)	

[a] Mann-Whitney U test, [b] Kruskal-Wallis test, *p-value<0.05

significant (p=0.353) (Table 2).

Discussion

This is the first report on the CNV in the SULT1A1 gene in Thai premenopausal breast cancer patients. Two copies of SULT1A1 genes was the most common genotype (33/34) in premenopausal Thai breast cancer patients undergoing TAM treatment, which corresponds to that reported in other populations, except for the four copies reported in Caucasian (Gjerde et al., 2010; Moyer et al., 2011) and Japanese populations (Yu et al., 2013). In contrast to a previous study (Gjerde et al., 2010), no association between the SULT1A1 copy number and plasma E2 concentration was found. However, this might reflect the low sample size and/or the very low prevalence of other copy numbers in the Thai breast cancer population.

The plasma E2 concentrations in these 34 premenopausal patients were highly variable and ranged from 415.0-4,186.5 pg/ml, which was higher than the previously reported E2 concentrations (Lum et al., 1997; Gjerde et al., 2008). These differences might come from several factors. Firstly, the premenopausal breast patients recruited in this study had naturally higher plasma E2 concentrations compared with the postmenopausal breast cancer patients included in the previous study (Gjerde et al., 2010). Secondly, the E2 concentration was determined by ELISA in this study and by radioimmunoassay in the previous study (Lum et al., 1997). Thirdly, the patients had been using TAM at 20 mg once daily for at least two months in this study compared to at 10 mg twice a day for an undefined period in the previous study (Lum et al., 1997). Finally, the larger number of patients recruited in this study (N=34) compared with the previous study (N=6) (Lum et al., 1997), and this additionally may include different phases of the menstrual cycle that would yield different plasma E2 concentrations. The plasma E2 concentration increases in the follicular phase until reaching a peak and then decreases in the ovulatory phase, increases again in the luteal phase then decreases to begin the new menstrual cycle. Although the menstrual cycle was not controlled in this study, the menstrual phase during each patient's menstrual cycle was predicted by interviewing them at the start of the study. Overall the plasma E2 concentrations were not significantly (p=0.195) different among the different menstrual phases (follicular phase (10/25), ovulatory phase (6/25) and luteal phase (9/25)) from those patients who informed their menstrual starting date in this study (data not shown).

An association between the plasma E2 concentration and TAM-associated ADRs was found in this study (p=0.017). The two TAM-associated ADRs in this preliminary study (irregular menstruation and vaginal discharge/dryness) might be a consequence of the estrogen antagonist effect of TAM. However, several factors need to be considered before reaching a firm conclusion. Firstly, the patient interview and medical record review were used to determine the TAM-associated ADRs in this study without using any other means of evaluation, and so the limitation of using retrospective or subjective data collection might have led to an over- or under-estimation of those ADRs. Other factors might need to be explored for controlling these possible confounders, such as the patient's level of follicle stimulating hormone and sex hormone-binding globulin and their individual E2 baseline concentrations. Secondly, the limited numbers of patients in this preliminary study could be problematic, especially as only three patients reported TAM-associated ADRs. Therefore, more patients need to be evaluated for this relationship before generalizing the result to the target population. Finally, those TAM-associated ADRs reported in this study did not include hot flashes, which are usually the most common TAM-associated ADRs in breast cancer patients.

In conclusion, these findings provide the preliminary prevalence of SULT1A1 CNV in Thai premenopausal breast cancer patients undergoing TAM treatment, including the association between the plasma E2 concentration and TAM-associated ADRs. The high prevalence of SULT1A1x2 copies was firstly suggested from this study, which indicated that most of the Thai premenopausal breast cancer patients are carrying SULT1A1 genes with normal enzyme activities.

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