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Frequency distribution of phenol sulfotransferase 1A1 activity in platelet cells from healthy Japanese subjects

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Abbreviations:
CYP, cytochrome P450; OHT, *trans*-4-hydroxytamoxifen; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PhIP, 2-amino-1-methyl-6-phenylimidazo (4,5-*b*) pyridine; SULT, sulfotransferase; TAM, tamoxifen

ABSTRACT

Aims: To determine the distribution of sulfotransferase 1A1 (SULT1A1) activities, we used *trans*-4-hydroxytamoxifen (OHT) as a substrate to test samples from a Japanese population to examine whether the *SULT1A1*2* allele can account for the wide distribution of OHT sulfating activity. We also studied genetic mutations other than the *SULT1A1*2* allele to determine the cause of differences in SULT1A1 protein expression and activity.
Methods: The subjects were 103 healthy Japanese adults. Identification of SULT1A1 genotypes was performed using a polymerase chain reaction-restriction fragment length polymorphism method. SULT1A1 activity in platelet cytosol was assayed using OHT as a substrate. SULT1A1 protein was detected using Western blotting analysis. Mutations other than *SULT1A1*2* in the *SULT1A1* gene were detected using sequencing analysis.
*Results: SULT1A1*2* allele frequency was found to be 16.5%, while SULT1A1 activity ranged from 63 to 1860 pmol sulfated/h/mg platelet protein (260 ± 241 pmol sulfated/h/mg platelet protein, median ± S.D.) using OHT as a substrate. The median values in subjects with *SULT*1/*2* (221 ± 113 pmol sulfated/h/mg platelet protein, range 63–442, $n = 26$) and *SULT*2/*2* (124 ± 66 pmol sulfated/h/mg platelet protein, range 74–231, $n = 4$) were significantly lower than that in subjects with *SULT*1/*1* (303 ± 267 pmol sulfated/h/mg platelet protein, range 97–1859, $n = 73$). A novel G148C mutation was found in one subject, who showed the lowest OHT sulfating activity, for a frequency of 0.49%.
Conclusion: There was wide variety of OHT sulfating activities found among the present healthy Japanese subjects. The *SULT1A1*2* allele was found to be a common variant allele and was associated with decreased OHT sulfating activity. These observations may be related to inter-individual variations of OHT pharmacokinetics and the pharmacologic effects of tamoxifen seen in Japanese patients with breast cancer.

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1. Introduction

SULT1A1 is a Phase II conjugating enzyme. Using 3′-phosphoadenosine 5′-phosphosulfate (PAPS) as a sulfate donor, the enzyme has been shown to sulfate lipophilic compounds hydroxylated mainly by cytochrome P450 (CYP) isozymes (Falany, 1991). Since SULT1A1 activity in the liver is correlated well with its levels in platelet cytosol, platelets have proven to be useful for evaluating inter-individual differences in SULT1A1 activity (Weinshilboum, 1990).

Price et al. (1989), Raftogianis et al. (1997), Brittelli et al. (1999), and Nowell et al. (2000), each studied the distribution of SULT1A1 activity using *p*-nitrophenol as a substrate and found wide individual variations. SULT1A1 is important not only for catalyzing the sulfating reaction of carcinogen, but also for metabolic detoxification and activation of therapeutic drugs. For example, this enzyme is involved in the metabolic pathways of acetaminophen (Ozawa et al., 1999; Lewis et al., 1996), *trans*-4-hydroxytamoxifen (OHT: detoxification) (Falany et al., 1994; Nishiyama et al., 2002; Chen et al., 2002), and minoxidil (activation) (Falany et al., 1994; Johnson et al., 1982). Platelet SULT1A1 activity correlates well with the urinary excretion of acetaminophen sulfate (Reiter and Weinshilboum, 1982). In addition, OHT concentrations in plasma and breast cancer tissue vary among breast cancer patients (MacCallum et al., 2000; Kisanga et al., 2004), and the level of SULT1A1 activity is considered to have an effect on the effectiveness of therapeutic drugs.

OHT is one of the active metabolites of tamoxifen (TAM) that is mainly produced by CYP2D6 (Crewe et al., 2002), and thereafter catalyzed by SULT1A1 and excreted (Falany et al., 1994; Nishiyama et al., 2002; Chen et al., 2002; Lien et al., 1989). Although OHT has been identified as one of the major active metabolites in human serum with a circulation level of less than 10% of that attained by the parent drug, it is approximately 100 times more interactive with human estrogen receptors than TAM (Kisanga et al., 2004; Katzenellenbogen et al., 1984; Lee et al., 2003). Therefore, we considered that establishment of a method for measuring OHT sulfating activity would be useful.

The *SULT1A1* gene encodes seven allozymes, *SULT1A1**1 to *7 (Li et al., 2001). There is a marked interethnic difference in the frequencies of SULT variants (Carlini et al., 2001). Especially, *SULT1A1**2 and *3 are more prevalent variants in Caucasian, Chinese and African–American populations. *SULT1A1**2 has been reported to be associated with low levels of 2-amino-1-methyl-6-phenylimidazo (4,5-*b*) pyridine (PhIP) sulfating activity, which lowers the risk of bladder cancer by suppressing metabolic activation of PhIP (Zheng et al., 2003). Likewise, various SULT1A1 activities or the *SULT1A1**2 allele have roles in OHT pharmacokinetics and TAM therapeutic effects. However, the effects of *SULT1A1**2 sulfating activity have been reported to be different according to the substrate (Ozawa et al., 1999). The dubious value of *p*-nitrophenol as a substrate for SULT activity has also been described earlier (Tabrett and Coughtrie, 2003). Therefore, the sulfating activity of *p*-nitrophenol might not necessarily reflect that of other substrates.

The *SULT1A1**2 allele has been found in Japanese subjects and shown to lower *p*-nitrophenol sulfating activity by using the recombinant SULT1A1 protein expressed in *Escherichia coli* (*E. coli*) (Ozawa et al., 1999). However, the distribution of SULT1A1 activity in a Japanese population has not been reported. In the present study, we investigated the distribution of SULT1A1 activities in Japanese subjects using OHT as a substrate. Our results clarified the influence of *SULT1A1**2 allele toward OHT sulfating activity using human platelet cytosol as the SULT1A1 enzyme source and a recombinant SULT1A1 protein expressed in *E. coli*. Further, we analyzed OHT sulfating activity on the basis of the specific amount of SULT1A1 protein and mutations other than *2 in the *SULT1A1* gene.

2. Materials and methods

2.1. Subjects and blood samples

One hundred three unrelated healthy Japanese subjects (45 males, 58 females) were randomly recruited from the Sekino Clinical Pharmacology Clinic (Tokyo, Japan). The mean ± S.D. age of the subjects was 28 ± 10 years old (range, 20–66 years). Clinical examination test results (i.e. white blood cells, red blood cells, platelets, haemoglobin, alanine aminotransferase, aspartate aminotransferase, triglyceride, and blood sugar) showed no abnormalities and none of the subjects were receiving concurrent medication. Venous blood sampling was always performed at 10:00 a.m. The subjects refrained from smoking and consuming alcohol for atleast 24 h prior, and also fasted, except for water, atleast 6 h prior to providing blood samples. Gender, age, and smoking habits were examined by questionnaire. All subjects gave written consent for participation in the study, after having been informed both verbally and in writing of the experimental procedure and purpose of the study. The protocol of the study was approved by the Ethics Committee of Sekino Clinical Pharmacology Clinic (Tokyo, Japan).

2.2. Platelet isolation and homogenization

Blood samples (7 ml) were collected in Vacutainer® tubes (containing EDTA-2Na as an anticoagulant, TERUMO, Tokyo, Japan) and centrifuged at 200 × *g* for 10 min. Each supernatant was centrifuged at 16,000 × *g* for 10 min at 4 °C, after which the platelet pellets were isolated and homogenized as described previously (Anderson and Weinshilboum, 1980; Van Loon and Weinshilboum, 1984). The total protein concentration in the platelet homogenates was measured with a BCA protein assay kit (PIERCE, Illinois, USA) using bovine serum albumin as the standard. The platelet homogenates were stored at below −80 °C until performing the enzyme reaction assays and Western blotting procedures.

2.3. SULT1A1 genotyping procedures

Genomic DNA was isolated using an SX-8G (Precision System Science, Chiba, Japan). Genotyping for ^{213}Arg/His (*2) and ^{223}Met/Val (*3) of the *SULT1A1* gene was performed