THE INFLUENCE OF ASIAN SPECIFIC VARIANT, CYP2D6*10 ON IN VITRO FORMATION OF ENDOXIFEN, AN ACTIVE METABOLITE OF TAMOXIFEN
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[Objective] Tamoxifen (TAM), a selective estrogen receptor modulator, is widely used in breast cancer adjuvant therapy. Plasma concentration of its secondary metabolite, endoxifen (EDX, 4-hydroxy-N-desmethyltamoxifen), may be a determining factor of TAM therapy due to the 100-fold higher in vitro antiestrogenic activity than TAM and the high plasma concentration. EDX plasma concentration was reported to be significantly low in poor metabolizers (PM) of CYP2D6, and thus should be attributed to less response to TAM therapy observed in this population. The objective of this study is to evaluate the metabolizing activity of CYP2D6*10, an Asian specific variant whose allele frequency is reported to be 40-50%, to produce EDX, and to evaluate the influence of having the variant on TAM therapy.

[Methods] TAM is metabolized to N-desmethyltamoxifen (NDM) by several CYPs and then converted to EDX exclusively by CYP2D6. NDM was incubated with baculovirus-expressed CYP2D6*1/*10 enzymes in the presence of NADPH regenerating system. Formation rates were evaluated by measuring EDX utilizing HPLC system equipped with a post-column in-line photoreactor prior to a fluorescence detector. Enzymatic activity of CYP2D6 was confirmed utilizing bufuralol metabolism as a control.

[Results] Formation rates of EDX from NDM were approximately 0.34 and 0.025 (pmol/min/pmol P450) for CYP2D6*1 and *10, respectively. The reaction was linear until 60min. The formation rate was 15-fold lower in CYP2D6*10. The response to TAM therapy of carriers of CYP2D6*10 would be as limited as PM. Since TAM metabolism involves other enzymes, more information using human microsome is to be determined in order to extrapolate this data to in vivo precisely.

FUNCTIONAL CHARACTERIZATION OF CYP2C19*16 IDENTIFIED FROM A JAPANESE SUBJECT
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Cytochrome P450 (CYP) 2C19 shows genetically determined polymorphism in humans and more than 10 variants gene have been reported. We previously found a novel single nucleotide alteration from a Japanese subject with a lowered capacity of CYP2C19-mediated 4'-hydroxylation after an oral administration of mephobarbital. This allele was newly designated as CYP2C19*16 coding CYP2C19.16 with R442C alteration. To assess the effect of R442C alteration on CYP2C19-mediated metabolism, we performed site-directed mutagenesis and cDNA expression in COS-1 cells and examined the kinetics of S-mephenytoin 4'-hydroxylation activity by CYP2C19.1 (wild-type) and CYP2C19.16. The western blotting showed equal expression of CYP2C19.1 and CYP2C19.16 at the protein levels. The Km value of CYP2C19.16 was 14-fold higher than that of CYP2C19.1. The Vmax and Vmax/Km values of CYP2C19.16 were 1.3- and 17-folds lower than those of CYP2C19.1, respectively. These results indicated that the R442C alteration decreased the CYP2C19-mediated S-mephenytoin 4'-hydroxylation. Taken together with our finding obtained from the in vivo study, it was suggested that individuals having CYP2C19*16 allele are likely to have lowered clearances of CYP2C19-mediated metabolism.