PURIFICATION OF CYTOCHROME P450 2B4 AND ITS CRYSTAL STRUCTURE IN COMPLEX WITH THE ANTIPLATELET DRUG CLOPIDOGREL
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[Purpose] Cytochromes P450 2B (CYP2B) catalyze the oxidation of a broad range of substrates including xenobiotics and steroids and serve as an excellent model for investigating structure-function relationships. A series of crystal structures of rabbit CYP2B4 have shown the large degree of structural flexibility in the presence of various imidazole based inhibitors. This study aimed to solve a structure of CYP2B4 in complex with the antiplatelet drug clopidogrel.

[Methods] The N-terminal modified and C-terminal His-tagged CYP2B4 with a H226Y mutation was expressed in E. coli and purified. Crystals were grown by sitting drop vapor diffusion using a commercially available kit. Data were collected at the Stanford Synchrotron Radiation Laboratory, and the structure was determined by molecular replacement using an ensemble of previously solved CYP2B4 structures.

[Results and Discussion] The Cα traces of the CYP2B4–clopidogrel complex revealed a compact, closed structure that resembles the conformation observed in the previously solved structure with the small molecule inhibitor 4-(4-chlorophenyl)imidazole. Clopidogrel occupied the active site with the chlorophenyl group closest to the heme. This ligand orientation is opposite to that expected based on metabolic data with human CYP2B6.

[Conclusions] These results suggest that adjusting both protein conformation and ligand orientation in the active site gives CYP2B4 the flexibility to bind the widest range of molecules.

EFFECTS OF POLYMORPHISMS IN CYP2D6 AND ABCC2 ON CLINICAL OUTCOMES OF ADJUVANT TAMOXIFEN THERAPY FOR BREAST CANCER PATIENTS
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[Purpose] The clinical efficacy of tamoxifen is suspected to be influenced by the activity of drug-metabolizing enzymes and transporters involved in the formation and elimination of its active forms. We investigated relations of polymorphisms in transporter genes as well as CYP2D6 to clinical outcome of patients with tamoxifen treatment.

[Method] The effects of CYP2D6 genotype and tag-SNPs of ABCB1, ABCC2 and ABCG2 on recurrence-free survival were investigated in 282 hormone receptor-positive breast cancer patients receiving tamoxifen monotherapy.

[Results] CYP2D6 variants were significantly associated with shorter recurrence-free survival (P=3.6×10⁻⁵; hazard ratio (HR) 9.52 [95% CI 2.79-32.45]). Among 51 tag-SNPs in transporter genes, a significant association was found at rs3740065 in ABCC2 (P=1.7×10⁻³; HR 10.64 [95% CI 1.44-78.88]). The number of risk alleles of CYP2D6 and ABCC2 showed cumulative effects on recurrence-free survival (P = 5.5×10⁻⁸). These results suggest that polymorphisms in CYP2D6 and ABCC2 are important predictors for the prognosis of breast cancer patients treated with tamoxifen.