FUNCTIONAL CHARACTERIZATION OF TWO NOVEL CYP2C19 VARIANTS (CYP2C19*18 AND CYP2C19*19) FOUND IN A JAPANESE POPULATION
Yumi Tsuneto1, Nobumitsu Hanioka1, Yoshiro Saito2, Tomoko Sumada1, Keiko Maekawa2, Keita Saito1, Jun-ichi Sawada2 and Shizuo Narimatsu1

1Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530 and 2Division of Biochemistry and Immunochemistry, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

CYP2C19 plays important roles in the metabolism of a wide range of therapeutic drugs and exhibits genetic polymorphisms with interindividual differences in metabolic activity. We have previously found two CYP2C19 allelic variants, CYP2C19*18 and CYP2C19*19 with R329H/I331V and S51G/I331V substitutions, respectively (H. Fukushima-Uesaka et al., Drug Metab. Pharmacokinet., 20, 300-307 (2005)). In this study, to investigate the effect of amino acid substitutions on CYP2C19 function, CYP2C19 proteins of wild-type (CYP2C19.1B) and variants (CYP2C19.18 and CYP2C19.19) were expressed in yeast cells, and their \(S\)-mephenytoin 4'-hydroxylation activities were determined. The \(K_m\) value of CYP2C19.19 for \(S\)-mephenytoin 4'-hydroxylation was significantly higher (3.0-fold) than that of CYP2C19.1B. Although no significant differences in \(V_{\text{max}}\) values on the basis of microsomal and functional CYP protein levels were observed between CYP2C19.1B and CYP2C19.19, the \(V_{\text{max}}/K_m\) values of CYP2C19.19 were significantly reduced to 29–47% of CYP2C19.1B. In contrast, the \(K_m\), \(V_{\text{max}}\) or \(V_{\text{max}}/K_m\) values of CYP2C19.18 were not affected by the corresponding amino acid substitutions. These results suggest that S51G substitution in CYP2C19.19 decreases the affinity toward \(S\)-mephenytoin of CYP2C19 enzyme, and that the genetic polymorphism of CYP2C19*19 also causes variations in the clinical response to drugs metabolized by CYP2C19.

STRAIN DIFFERENCE OF IN VIVO AND IN VITRO METABOLISM OF ZALEPLON IN RATS
Chiaki Tanoue1, Manami Hirata1, Kazumi Sugihara1, Shigeyuki Kitamura1,2 and Shigeru Ohta1

1Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553 and 2Nihon Pharmaceutical University, 10281 Komuro, Saitama 362-0806, Japan

Zaleplon (ZAL; N-[3-(3-cyanopyrazolo[1,5-a]pyrimidin-7-yl)-N-ethyl]acetamide) is a non-benzodiazepine sedative-hypnotic agent for the treatment of insomnia. Preclinical studies have shown that the major metabolite of ZAL in humans and monkeys is 5-oxo-Zaleplon (5-oxo-ZAL), an aromatic ring oxidation product at the position adjacent to the nitrogen atom of the pyrimidine ring, while in rats, it is N-desethyl-Zaleplon (N-desethyl-ZAL), a side chain oxidation product. Studies with human liver preparations have indicated that ZAL is metabolized to 5-oxo-ZAL by cytosolic aldehyde oxidase (AO). NADPH-dependent metabolism of ZAL to N-desethyl-ZAL with microsomal cytochrome P450 (CYP) has also been observed. In human liver microsomes, N-desethyl-ZAL formation was predominantly catalyzed by CYP3A forms. It may be presumed that the marked species differences of the metabolic process are due to differences in AO activity. In this study, we compared the in vivo and in vitro metabolism of ZAL by two strains of rats, Sea:SD (high AO activity) and WKA/Sea (low AO activity). The activity for metabolism of ZAL to 5-oxo-ZAL by liver cytosol of Sea:SD rats was 20-fold higher than that of WKA/Sea rats. However, no difference in the metabolism of ZAL to N-desethyl-ZAL by liver microsomes in the presence of NADPH was observed. On the other hand, when ZAL was orally administered to these rats at 50 mg/kg, a marked strain difference was found in the metabolism of ZAL; the major metabolite in plasma of Sea:SD rats was 5-oxo-ZAL, while in plasma of WKA/Sea rats, it was N-desethyl-ZAL. It appears that the high recovery of N-desethyl-ZAL in WKA/Sea rats was caused by metabolic switching.