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MECHANISM-BASED INHIBITION OF CYP2D6 BY PAROXETINE. K.M. Bertelsen, K. Venkatakrishnan, L.L. von Moltke, R. Scott Obach, and D.J. Greenblatt. Tufts University, Boston, MA and Pfizer Inc., Groton, CT.

The selective serotonin reuptake inhibitor paroxetine (PX) is a potent CYP2D6 inhibitor, but the mechanism of inhibition is not established. Inhibition of CYP2D6 in vitro by PX, fluoxetine (FLU) and quinidine (Q) was studied in human liver microsomes (HLM) using dextromethorphan O-demethylation (25 μM) as the index reaction. Without preincubation of HLM with inhibitor, mean IC_{50} values were: 2.5 μM for PX, 0.2 μM for FLU, and 0.23 μM for Q. Preincubation of inhibitor with HLM significantly reduced the PX IC_{50} by eight-fold to 0.3 μM; in contrast, the IC_{50} for FLU and Q were actually increased (to 0.42 μM and 0.36 μM, respectively). PX produced time-dependent inactivation, with an apparent rate constant of 0.17/min based on a Kitz-Wilson plot. This was not true for FLU or Q. Incubation of PX with recombinant CYP2D6 yielded a difference spectrum with maximum absorbance at 456 nm, and a time-dependent increase in absorbance, consistent with inactivation via formation of a carbene-heme complex. Thus PX, unlike FLU and Q, appears to inhibit CYP2D6 at least in part through a mechanism-based process, probably attributable to the methylenedioxy substituent of PX.

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PHARMACOGENOMIC STUDIES USING PARAFFIN EMBEDDED TUMOR SAMPLES. J. M. Rae, PhD, J. Scheys, BS, K. E. Cordero, BS, M. D. Johnson, PhD, University of Michigan, Georgetown University, Ann Arbor, MI.

Paraffin embedded tumor samples are valuable in the study of cancer. Besides use in routine staging and marker analysis, they are used to test new prognostic and predictive markers. However, their use in retrospective pharmacogenomic analysis of clinical trials has yet to be evaluated. We set out to establish genotyping methods for polymorphic CYP450s in formalin-fixed archival tumor samples and determine if fixation, processing or somatic changes in the tumor affect our ability to determine germ-line mutations. To establish the assay, paraffin blocks were made from 8 cell lines. DNA from cell cultures and paraffin sections was purified using the Qiagen system. These samples were genotyped for CYP2C8, CYP2C9, CYP2C19 and CYP2D6 alleles using PCR-RFLP. This allowed optimization of DNA extraction and genotyping for these materials and showed that fixation and processing did not significantly alter the genotypes obtained (>97% concordance: cell lines vs paraffin sections). Next, sections were cut from archival tumor blocks from 10 patients for whom gDNA from peripheral blood was available. Again, concordance was very high with the same genotype being obtained for 97% of the alleles tested. Conclusions: accurate genetic testing for polymorphisms on CYP2Cs and CYP2D6 can be obtained from archival paraffin embedded tumor samples. Thus, pharmacogenetic analysis can be applied to existing cancer therapy trials to test associations between these polymorphisms and treatment response.

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SULFOTRANSFERASE (SULT) 1A1 PHARMACOGENETICS: FUNCTIONAL 5'-FLANKING REGION (5'-FR) POLYMORPHISMS. J. Prondzinski, B. Thomae, L. Wang, B. Eckloff, E. Wieben, PhD, R. Weinshilboum, MD, Mayo Clinic, Mayo Graduate School-Mayo Clinic, Rochester, MN.

SULT1A1 catalyzes the sulfate conjugation of many drugs, xenobiotics and hormones. Human platelet SULT1A1 activity and thermal stability vary widely among individuals and are regulated by inheritance. A single nucleotide polymorphism (SNP) in the *SULT1A1* open reading frame (ORF) (Arg213His) is associated with low levels of enzyme activity and thermal stability. However, not all SULT1A1 phenotypic variation is explained by this SNP. We set out to determine whether polymorphisms in the 5'-FR also influence SULT1A1 expression. We resequenced 2 kb of the *SULT1A1* 5'-FR using 32 DNA samples selected for extreme platelet activity and thermal stability. Three common SNPs were observed (-1985 C/G, -624 C/G, -397 G/A – numbered relative to the "A" in the ATG codon). The -624 and -397 SNPs were associated with *SULT1A1* ORF genotype and with platelet SULT1A1 phenotype. Luciferase reporter gene constructs containing these SNPs were used to transfect HepG2 cells. There was a 59% average decrease in activity (N=9) for the construct containing (-624, -397) GA as compared with CG, and a 90.5% average decrease in activity (N=9) for a shorter construct that contained only (-397) A vs. G. These results indicate that 5'-FR genetic polymorphisms may contribute to SULT1A1 pharmacogenetic variation and that inherited variation in the regulation of transcription could influence sulfate conjugation catalyzed by this isoform.

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INFLUENCE OF CYP2D6 GENOTYPE ON THE QTc INTERVAL AND PLASMA CONCENTRATIONS OF THIORIDAZINE AND ITS METABOLITES IN PSYCHIATRIC PATIENTS TAKING CHRONIC THERAPY. R. Thanacoody, MRCP, A. K. Daly, PhD, S. H. Thomas, MD, University of Newcastle, Newcastle, United Kingdom (Great Britain).

Purpose. The antipsychotic agent thioridazine is an important cause of QTc interval prolongation and torsade de pointes and CYP2D6 is important in its metabolism. This study was performed to determine whether CYP2D6 genotype influences the QTc interval in long-term recipients of thioridazine.

Methods: Patients receiving stable doses of thioridazine were recruited from psychiatric hospitals and clinics. A 12-lead ECG was recorded and the QTc interval measured using a digitiser. CYP2D6 genotyping was performed for 4 common allelic variants associated with absence of activity (*3, *4, *5 and *6). When available, plasma was analysed for concentrations of thioridazine and its metabolites, thioridazine-2-sulfoxide (T-2SO), thioridazine-2-sulfone (T-SO2), and thioridazine-5-sulfoxide (T-5SO) using high performance liquid chromatography.

Results: Genotyping indicated that the 93 patients studied included 54 extensive metabolizers (EM), 30 intermediate metabolizers (IM, heterozygotes with 1 mutant allele) and 9 poor metabolizers (PM, 2 mutant alleles). The mean doses of thioridazine taken in the previous 24 h (104 ±122, 113 ± 86, and 115±185 mg respectively) and mean QTc intervals (425 ± 29, 427 ± 22, 411 ± 41 ms respectively) were similar for the 3 groups. For the 30 subjects for whom data were available, QTc intervals were significantly correlated with plasma concentrations of thioridazine, T-2SO and T-SO2 but not T-5SO. Mean thioridazine and metabolite concentrations did not differ significantly between EM, IM and PM subjects, although the numbers involved were small (15, 12 and 3 respectively).

Conclusions: This study does not suggest that CYP2D6 genotype substantially affects risk of thioridazine induced QTc prolongation.