29. Aldehyde oxidases and other molybdenum hydroxylases

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Drug oxidation (and reduction) mediated by the molybdenum containing enzyme, aldehyde oxidase (AO), is often overlooked in comparison to reactions catalyzed by cytochrome P450. However, aldehyde oxidase and the closely related xanthine oxidoreductase (XOR) catalyse the oxidation of a wide range of N-heterocyclic drugs in addition to aldehyde oxidation and reductive reactions. Typically, in vivo conversion of drugs to AO-generated metabolites is rapid and metabolites are excreted directly without conjugation. Consequently, drugs may be quickly inactivated or bioavailability may be reduced due to AO or XOR-catalysed oxidation in liver (AO and XOR) or gut (XOR). It is difficult to express AO and XOR activity in recombinant systems and until recently, information on these enzymes has predominantly come from in vitro animal studies. This has provided valuable data on substrate/inhibitor specificity and possible clinical implications. Many in vitro AO inhibitors have been identified but, to date, there is little clinical indication that inhibitory drug interactions are significant in vivo. In contrast, co-administration of XOR inhibitors can be used to modulate drug therapeutics. Recently, full elucidation of the crystal structure of bovine milk XOR and Desulfovibria gigas AO and determination of their gene structure has expanded our knowledge of these enzymes considerably. AO and XOR each have a single functional gene in human liver (hAOX1 and XOR) but, unlike XOR, the AO gene is not conserved in all species and thus results from animal studies have to be interpreted with caution. Human AO and XOR genes appear to be controlled by complex but differential mechanisms at both transcriptional and post-translational levels. For example, studies in mice have indicated that both enzymes may be induced via the aryl hydrocarbon receptor (AhR) pathway, however, there is no clinical evidence that metabolism of drugs catalyzed by these enzymes is altered in smokers or during exposure to other AhR inducers. There are conflicting reports on the implications of AO polymorphism but some preliminary studies have indicated that human AO single nucleotide polymorphisms (SNPs) can influence drug efficacy whereas XOR regulation and polymorphism may be more important in various physiological and pathophysiological mechanisms.

30. Flavin-Containing monooxygenases

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The flavin-containing monoxygenases (FMOs) oxidatively metabolize numerous toxicants and approximately 2% of clinically relevant drugs. In the human, 5 *FMO* genes encode functional enzymes (FMO1-5) with FMO1, 2 and 3 being most important for drug and toxicant metabolism. FMO1 is expressed at high levels in the fetal liver, small intestine and kidney while FMO3 is expressed at high levels in the adult liver. FMO2 is primarily a lung-specific enzyme, but its impact is minimized by a premature stop codon common in populations outside of Africa. In the human, FMO1 is expressed at its highest level in the 1st trimester fetal liver, then declines and is silenced a few days after birth. In contrast, FMO3 is essentially absent in the fetal and neonatal liver, but is detectable in most individuals by 1 to 2 years of age. Intermediate expression is observed in individuals between ages 2 and 11. Adult expression is usually seen by age 18. FMO3 promoter analyses revealed the presence of an NFY (position -75 to -59), Pbx_2/HOX (position -115 to -103), HNF4α (position -167 to -152), YY1 (position -258 to -248) and C/EBPβ (position -456 to -444) responsive elements. The NFY, HNF4α and C/EBPβ sites appear most important for constitutive expression in the adult while developmental changes in the C/EBPβ LAP:LIP ratio likely are involved in regulating FMO3 developmental expression. Several functional *FMO3* variants have been identified. Hypomorphic variants have been associated with increased sulindac chemoprevention efficacy in familial adenomatous polyposis (FAP) patients. However, preliminary analysis of data from a recently completed phase III randomized placebo-controlled trial in which patients with sporadic colorectal polyps received a combination of difluoromethylornithine (DFMO) and sulindac (N=191) or placebo (N=184) do not appear to corroborate these earlier findings. A significant association was observed with decreased colonic mucosa PGE_2

levels and the FMO3 G308 variant (rs2266780) (P=0.038) and increased colonic mucosa putrescine levels and the FMO3 K158 variant (rs2266782) (P=0.036). However, no association was observed between the FMO3 variants and risk of adenoma recurrence. These contradictory data may be explained by the recently discovered opposing actions of FMO3. Consistent with earlier studies, hypomorphic FMO3 variants would favor a cyclooxygenase-dependent antitumor effect, but hypermorphic variants would favor an antitumor effect through the ability of sulindac sulfoxide and sulfone to enhance SAT1 expression through PPARγ and reduce cellular polyamine levels via the SLC3A2 transporter.

31. UDP-glucuronosyl transferases

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Symposium 7: Scientific and Regulatory Perspectives on Non-CYP Drug Metabolizing Enzymes and Transporters UDP-glucuronosyl Transferases Gross Gerhard, AstraZeneca R & D, Alderley Park, Macclesfield, UK UPD-glucosyl transferases are a superfamily of endoplasmatic reticulum bounded enzymes which belong to the so called phase II enzymes. They catalyse the attachment of glycolsyl groups (glucuronic acid, glucose and xylose) to an acceptor molecule, the so called aglycone. There is a great variety of functional groups which may function as attachment group e.g. any alcohol, aromatic as well as aliphatic; carboxylic acids, thiols, amines and acidic carbon atoms. Most common reaction is the transfer of a glucuronic acid moiety to an aglykone. This process is called glucuronidation. The group of enzymes catalyzing this reaction is referred to as UGTs. UGTs are divided into two families UGT1 and UGT2. For human 31 genes have been identified expressing UGTs including some pseudogenes. The most important human UGTs are: UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6 as well as UGTs 1A7, 1A8, 1A9, 1A10; from the UGT1 family and UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15 and UGT2B17 from the UGT2 family. These enzymes are expressed mainly in the alimentary system, liver, kidney, brain, lung as well as (low level expression) in steroidogenic tissues like breast, prostate and adrenal. They play a significant role in metabolism of many endogenous (bilirubine, bile acids and steroids) and xenobiotic compounds. Glucuronidation converts a substrate/aglykon to a more polar and water soluble form and therefore facilitates its elimination through the kidney via glomerular filtration or active secretion as well as active transport through bile into small intestine. Genetic polymorphism has been reported for quite a few UGTs e.g. UGT1A1 (polymorphisms of this enzyme also relevant for Criegler-Najjar syndrome and Gilberts disease), UGT1A6, UGT1A7, UGT2B4, UGT2B7 and UGT2B15. Functional significance so far seems to be only clearly proven for UGT1A1. Nuclear receptors are able to regulate UGT expression, e.g. UGT1A1 induction through PXR and CAR. Major relevant aspects of UGTs and glucuronides for drug development are basically all factors contributing to safety and efficacy of a drug: drug/drug interactions; impact of genetic polymorphism of UGTs; interplay e.g. metabolic switching of CYP mediated metabolism with UGT metabolism as well as interactions with transporter. Acyl glucuronides are in particular seen as relevant for safety. Case studies will be presented to illustrate and discuss all these points. Regulatory guidance's, where glucuronides are explicitly mentioned or of relevance are discussed also.

32. Abstract not available

33. Abstract not available

34. A Systematic analysis of predicted phosphorylation sites within the human PXR protein

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The pregnane x receptor (PXR, NR1I2) regulates the expression of genes that encode drug metabolizing enzymes and drug transporter proteins in liver and intestine. Understanding the molecular mechanisms that modulate PXR activity is therefore critical for the development of effective therapeutic strategies. Several recent studies have implicated activation of kinase signaling pathways in the regulation of PXR biological activity, though direct evidence and molecular mechanisms are currently lacking. We therefore sought to characterize potential phosphorylation sites within the PXR protein using