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DASATINIB (SPRYCEL) AND IMATINIB MESYLATE (GLEEVEC): SIMILAR TARGETS AND THERAPEUTIC INDICATION, DISSIMILAR SELECTIVITY PROFILES AND IN VITRO CARDIOTOXIC POTENTIAL. V. Sasseville, H.S. Fang, M. Slade, S. Wells, J. Canale, J. Megill, B. Grubor, A. Fletcher, F. Lee, W.J. Freebern. Bristol Myers Squibb Research and Development, Syracuse, NY and Princeton, NJ.

Imatinib mesylate (Gleevec), a tyrosine kinase (TK) inhibitor indicated for chronic myelogenous leukemia (CML), is associated with congestive heart failure (CHF). Ultrastructural mitochondrial abnormalities were reported from both imatinib-treated mice and CML patients with CHF, which correlated with collapsed mitochondrial membrane potential, reduced cell viability, and increased apoptosis in imatinib-treated rat cardiomyocytes in vitro (Kerkela et al., Nat. Med. 2006). Dasatinib (Sprycel), a TK inhibitor with a different kinase selectivity profile than imatinib, has been approved for use in CML patients. It was postulated that TK inhibition is responsible for imatinib cardiotoxicity, and since dasatinib is a much more potent TK inhibitor than imatinib, at respective pharmacologically relevant concentrations, the in vitro cardiotoxicological potential of these drugs at pharmacologically-relevant concentrations was investigated in primary rat cardiomyocytes. Dasatinib did not significantly affect mitochondrial membrane potential, cell viability, apoptosis, or cellular ultrastructure in vitro, whereas imatinib significantly affected these parameters. To investigate possibilities as to why imatinib is more directly cardiotoxic than dasatinib, the activation status of signaling components downstream to molecular targets of these drugs was investigated. Luminex"s xMap" technology results demonstrated that the phosphorylation status of both ERK and MEK were increased in imatinib treated cells and were either not different from control or decreased in dasatinib treated cardiomyocytes. Interestingly, a link to cardiovascular disease and activation of MEK signaling has been reported (Baba et al. Cardiovasc Res. 2003). The question, and therefore a challenging research opportunity, remains as to what comes first, MEK signaling activation or cardiovascular dysfunction.

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PULSED AND CONTINUOUS PATTERNS OF DIACETYL (2,3-BUTANEDIONE) INHALATION CAUSE RHINITIS, LARYNGITIS, TRACHEITIS, AND BRONCHITIS IN RATS.

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Diacetyl is an alpha-diketone imparting the flavor of butter to food. During microwave popcorn and flavoring production, some workers inhaling diacetyl-containing vapor mixtures develop fixed airways obstruction. Workplace studies demonstrate large diacetyl air concentration fluctuations. To assess the airway toxicity of diacetyl and the use of time-weighted averages (TWAs) as the exposure metric, rats inhaled diacetyl at comparable TWAs over 6 hours, delivered either continuously or as four ~15 minute pulses. The target concentrations for the four ~15 minute pulses were 600, 1200, and 1800 ppm. The resulting 6 hour TWAs were 122 (low), 225 (medium), or 365 (high) ppm. Rats in the continuous groups inhaled diacetyl continuously over 6 hours, producing TWAs of 120 (low), 224 (medium) or 356 (high) ppm. An additional group, the single pulse group, inhaled one high (~1800 ppm) diacetyl pulse (92.9 ppm 6 hour TWA). Diacetyl caused concentration-dependent epithelial necrosis and neutrophilic inflammation in the nose, larynx, trachea and bronchus. Significant bronchial epithelial damage was observed only in the high exposure groups. In the first nasal section, the continuous pattern of low exposure caused greater damage than the pulsed low exposure. In remaining sections and concentrations, the pathology score was unaffected by exposure pattern. The single high pulse diacetyl exposure also caused significant necrosis and/or inflammation in the first nasal section. These findings suggest that at concentrations exceeding 100 ppm, TWAs are a reasonable measure for diacetyl exposures. However, a single, pulse, high diacetyl exposure can cause respiratory epithelial injury.

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DEVELOPMENT AND EVALUATION OF GENE EXPRESSION-BASED SIGNATURES PREDICTIVE AND DIAGNOSTIC OF BILE DUCT HYPERPLASIA IN RATS. E. Blomme, Y. Yang, R. Brennan, B. Jeffy. Abbott Laboratories, Abbott Park, IL. Iconix Biosciences, Mountain View, CA.

Bile duct hyperplasia (BDH) occurs after days or weeks of exposure to various chemicals and is typically identified late in drug discovery. For this reason, a predictive biomarker of BDH would be of significant value in drug discovery. Gene expression-based signatures have been shown to predict with reasonable accuracy the development of various pathologic findings. In this study, we evaluated whether predictive and diagnostic gene expression signatures could be generated to predict or confirm BDH in rats. Using liver gene expression profiles from rats exposed to reference compounds (extracted from DrugMatrix®), we derived 2 gene expression signatures for BDH in rats. The predictive signature was designed to predict, after 1 to 5 days of dosing, the occurrence of BDH after weeks of continuous dosing. The diagnostic signature was designed to correlate with the presence of BDH after 5 days of dosing. To further characterize the performance characteristics of these 2 signatures, forward validation was conducted. Male rats were treated for 1, 5, or 28 days with 8 compounds not used to generate the 2 signatures. Liver gene expression profiles from the 1 and 5 day time points were generated and liver histopathology was assessed for all time points. The diagnostic signature had an accuracy of 69% (sensitivity 100%; specificity 62%), while the predictive signature had an accuracy of 88% (sensitivity 90%; specificity 83%). Interestingly, the diagnostic signature had excellent predictive properties, correctly identifying the 5 compounds that induced BDH after 28 days. In conclusion, these data suggest that these signatures can reliably identify compounds inducing BDH at an early stage in drug discovery.

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TOXICOGENOMIC AND MORPHOLOGIC CORRELATES OF ADAPTIVE HEPATOCELLULAR HYPERTROPHY IN RATS. P. Caplazi, M. Irwin, M. Cooper, M. Albassam, S. Platz and K. Kolaja. Roche Palo Alto, Palo Alto, CA.

Adaptive hepatocellular enlargement occurs in rats exposed to certain xenobiotics. While the underlying mechanisms may differ, microscopic findings are often similar and non-specific. Elucidation of mechanisms, crucial for assessment of toxicological relevance, therefore requires additional tests. Gene expression profiles can highlight major metabolic pathways and might yield insight into pathogenetic processes. This study describes an example of coordinated morphologic and toxicogenomic evaluation of hepatocellular hypertrophy. Dose-dependent, severe hepatomegaly occurred in a two-week rat oral range-finding toxicity study. Serum chemistry was unrevealing with mildly elevated GGT at high dose. Other abnormalities, limited to females, included mild hyperproteinemia, hyperalbuminemia, hypercholesterolemia and hypertriglyceridemia. Microscopically, diffuse hepatocellular hypertrophy was observed in the absence of distinguishing features. Toxicogenomic (TGX) evaluation indicated activation of constitutive androstane receptor (CAR) and pregnane-X-receptor (PXR), including induction of Phase I and II metabolizing enzymes. TGX provided no evidence of arylhydrocarbon receptor (AhR) or peroxisome-proliferator activated receptor (PPAR)-mediated mechanisms of hypertrophy. Accordingly, peroxisome proliferation did not account for hepatocellular hypertrophy as shown by unremarkable immunohistochemistry for peroxisome membrane protein. Consistent with CAR activation, electron microscopy (EM) suggested increase of smooth endoplasmic reticulum, resulting in occasional separation of the otherwise packed and numerous mitochondria in some hepatocytes, a feature not seen in controls. EM further suggested slight increase of RER, which may accompany cellular hyperplasia and is therefore consistent with PXR activation; however, quantitative evidence for hyperplasia was not established by light microscopy. The data presented herein reveal how TGX and pathology can be combined to better understand the altered pathways and mechanisms that result in morphological alterations.