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ABC gene-ranking for prediction of drug-induced cholestasis in rats

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ABSTRACT

As legacy toxicogenomics databases have become available, improved data mining approaches are now key to extracting and visualizing subtle relationships between toxicants and gene expression. In the present study, a novel "aggregating bundles of clusters" (ABC) procedure was applied to separate cholestatic from non-cholestatic drugs and model toxicants in the Johnson & Johnson (Janssen) rat liver toxicogenomics database [3]. Drug-induced cholestasis is an important issue, particularly when a new compound enters the market with this liability, with standard preclinical models often mispredicting this toxicity. Three well-characterized cholestasis-responsive genes (Cyp7a1, Mrp3 and Bsep) were chosen from a previous in-house Janssen gene expression signature; these three genes show differing, non-redundant responses across the 90+ paradigm compounds in our database. Using the ABC procedure, extraneous contributions were minimized in comparisons of compound gene responses. All genes were assigned weights proportional to their correlations with Cyp7a1, Mrp3 and Bsep, and a resampling technique was used to derive a stable measure of compound similarity. The compounds that were known to be associated with rat cholestasis generally had small values of this measure relative to each other but also had large values of this measure relative to non-cholestatic compounds. Visualization of the data with the ABC-derived signature showed a very tight, essentially identically behaving cluster of robust human cholestatic drugs and experimental cholestatic toxicants (ethinyl estradiol, LPS, ANIT and methylene dianiline, disulfiram, naltrexone, methapyrilene, phenacetin, alpha-methyl dopa, flutamide, the NSAIDs––indomethacin, flurbiprofen, diclofenac, flufenamic acid, sulindac, and nimesulide, butylated hydroxytoluene, piperonyl butoxide, and bromobenzene), some slightly less active compounds (3′-acetamidofluorene, amsacrine, hydralazine, tannic acid), some drugs that behaved very differently, and were distinct from both non-cholestatic and cholestatic drugs (ketoconazole, dipyridamole, cyproheptadine and aniline), and many postulated human cholestatic drugs that in rat showed no evidence of cholestasis (chlorpromazine, erythromycin, niacin, captopril, dapsone, rifampicin, glibenclamide, simvastatin, furosemide, tamoxifen, and sulfamethoxazole). Most of these latter drugs were noted previously by other groups as showing cholestasis only in humans. The results of this work suggest that the ABC procedure and similar statistical approaches can be instrumental in combining data to compare toxicants across toxicogenomics databases, extract similarities among responses and reduce unexplained data varation.

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1. Introduction

Cholestasis, or the reduction of bile flow, can progress to serious hepatotoxicity in patients and is a particular concern in evaluation of novel drug candidates. Extrahepatic cholestasis occurs within the bile duct, forming blockages via concentrated, precipitated drugs, or by damage of the biliary epithelial cells, and loss of bile transport and flow. Although a variety of drugs are concentrated in the bile, drug-induced obstructive cholestasis is often readily observed preclinically, and can be avoided. Intrahepatic cholestasis associated with hepatotoxicity generally results from bile salt export pump (BSEP) inhibition, which if not adequately handled by accessory pathways such as MRP3, results in accumulation of detergent-like bile salts, that are toxic to hepatocytes. At high dose levels, novel drug candidates can bind to multiple hepatic transporters and trigger cholestatic signals. Therefore, screening for transporter inhibition in membrane vesicles is a typical step in the drug discovery and development process [21,22,25,33]. Identification and translational understanding of cholestatic responses in preclinical species is key step in the nonclinical safety assessment before initiating pharmaceutical clinical development.

The rat is a well-characterized model in which to study cholestasis, and is a principal species for preclinical toxicology studies. There are some differences from human: bile salts are conjugated by different enzymes (cholic acid and chenodeoxycholic acid are predominantly conjugated with glycine in man versus with taurine in the rat), the molecular weight "filter" for drug biliary secretion is generally lower than in humans (~350 molecular weight in rat, versus ~500 in humans), and the rat lacks a gall bladder [1,17]. This latter difference is an experimental advantage in that there is more consistent bile flow regulation in rats. Additionally, laboratory rats eat a well-defined, carbohydrate-rich rodent chow and feed most heavily during the dark cycle, more continually through the day than most humans. These feeding habits contribute to less variation in rat biliary transport and a continual and higher volume of bile flow [10]. Despite numerous species differences when comparing drug effects in rat to human, most transporter inhibition studies show remarkable consistency; for example, no rat versus human differences were observed in a large study of drug inhibition of BSEP transport [21].

A number of models of cholestasis in the rat are well studied and established: biliary ligation, which models bile duct obstruction; ANIT, which damages/destroys biliary epithelium; LPS, which down-regulates much of the hepatic metabolism including most transporters; and glucuronidated ethinyl estradiol-induced cholestasis by inhibition of BSEP [2,7,11,20,23,34,36,39]. All of these models have been characterized by gene expression on microarrays [7,11,34,39]. There is a misleading tendency to generalize the specifics of each of these different models to all forms of cholestasis. For characterization of novel drug candidates, there is a need to capture as much of the invariant cholestasis gene response as possible across a wide range of pharmaceutical structural classes.

At Janssen we have developed a predictive, 24 h treatment rat toxicogenomic database using approximately one hundred non-proprietary treatments and four times as many proprietary compound treatments, the latter having supporting exposure, clinical chemistry and histopathology data, often out to a month of dosing. We have published predictive gene signatures for non-genotoxic carcinogens [24,27] macrophage and PPARα receptor activation [18] and particularly oxidative stress/reactive metabolite responses for detecting idiosyncratic hepatotoxicants [14,15]. We previously developed a cholestasis gene expression signature using in-house proprietary compounds that induced cholestasis in rat studies (unpublished data). In the present study, we have used three robust non-redundant genes (Cyp7a1, Mrp3 and Bsep) from this previous rat signature which have been implicated in cholestasis [13,19,29,35]. Non-redundant genes differentially respond with large variances to compounds across our database, therefore a single one of these genes suffices for relevant transcriptomics information. Protein expression of MRP3 is up-regulated during cholestasis and genetic defects in MRP3 have been linked with pregnancy or estrogen hormone-induced cholestasis [28]. Mutations of the gene encoding the human bile salt export pump are implicated in progressive familial intrahepatic cholestasis type 2 (PFIC2), and inhibition of human and/or rat BSEP transporter constructs have been demonstrated to be correlated to cholestatic potential [4]. CYP7A1 is an important participant in transcriptional regulation, due to bile acid synthesis via nuclear hormone receptors and modulates cholestasis response via the classical pathway of bile enzyme synthesis [12,26,31].

From this starting point of three genes, we then applied the "aggregating bundles of clusters" (ABC) statistical approach [3] to develop a rat cholestasis gene expression signature that better discriminates cholestatic from non-cholestatic compounds. Both our unpublished proprietary signature and the present 100-gene rat cholestasis signature of this work yield good separation of compounds, and can discriminate many drugs known to cause cholestasis in man.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats, seven to eight weeks old, and approximately 275 g body weight (Charles River Laboratories, Inc.) were used for experiments. Animals were individually housed in wire-bottom cages, on a 12 h/light/dark cycle, and fed Certified Rodent Diet 5002 (LabDiet) ad libitum, with free access to water. On the day prior to dosing, animals were randomized by weight and allocated to groups ($n = 3$ rats/group). The route of administration for each test article is denoted in Table 1. Oral gavage was the most commonly utilized mode of administration as it is the typical route for the majority of compounds (predominantly pharmaceuticals) included in the training set. Animals were dosed in the morning hours, with health checks performed at 1 h and 4 h after dosing, and the end of the workday. Any animals deemed to be in poor health status were evaluated by a veterinarian: in these experiments no animals were prematurely terminated due to poor health.

Necropsy was performed on fasted rats, 24 h following dosing. Rats were killed by exsanguination, severing the vena cava under CO_2 analgesia, and liver sections (approximately 200 mg of the right medial lobe) were transfered to labeled cryo tubes and snap frozen in liquid nitrogen.

The selected dose level was the maximum tolerated dose, as elucidated from the literature [18,24]. Compound selection rationale, dose levels, route of exposure, group size, and experiment number are documented within Johnson and Johnson publically-accessible NIEHS-hosted database http://cebs.niehs.nih.gov.

In all instances, the animals were humanely handled and according to institutional guidelines, in accordance with the IACUC and NRC Guide for the Care and Use of Laboratory Animals.

2.2. Generation of microarray data

Total RNA was extracted from liver samples using Qiagen RNEasy Midi kits (Qiagen, Inc., Valencia, CA) as per kit instructions. The amount of RNA in the samples was determined spectrophotometrically by absorbance ratio at 260 and 280 nm. Quality of RNA in the samples was assessed using rRNA peaks determined by an Agilent 2100 Bioanalyzer.