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In vitro evaluation of hepatotoxic drugs in human hepatocytes from multiple donors: Identification of P450 activity as a potential risk factor for drug-induced liver injuries

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ABSTRACT

A possible risk factor for drug-induced hepatotoxicity is drug metabolizing enzyme activity, which is known to vary among individuals due to genetic (genetic polymorphism) and environmental factors (environmental pollutants, foods, and medications that are inhibitors or inducers of drug metabolizing enzymes). We hypothesize that hepatic cytochrome P450-dependent monooxygenase (CYP) activity is one of the key risk factors for drug induced liver injuries (DILI) in the human population, especially for drugs that are metabolically activated to cytotoxic/reactive metabolites. Human hepatocytes from 19 donors were evaluated for the activities of 8 major P450 isoforms: CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4. Extensive individual variations were observed, consistent with what is known to be in the human population. As CYP3A4 is known to be one of the most important P450 isoforms for drug metabolism, studies were performed to evaluate the relationship between the *in vitro* cytotoxicity of hepatotoxic drugs and CYP3A4 activity. In a proof of concept study, hepatocytes from six donors (lots) representing the observed range of CYP3A4 activities were chosen for the evaluation of *in vitro* hepatotoxicity of four drugs known to be associated with acute liver failure: acetaminophen, cyclophosphamide, ketoconazole, and tamoxifen. The hepatocytes were cultured in collagen-coated plates and treated with the hepatotoxicants for approximately 24 h, followed by viability determination based on cellular adenosine triphosphate (ATP) contents. HH1023, the lot of hepatocytes with the highest CYP3A4 activity, was found to be the most sensitive to the cytotoxicity of all 4 hepatotoxic drugs, thereby suggesting that high CYP3A4 activity may be a risk factor. To further validate the relationship, a second study was performed with hepatocytes from 16 donors. In this study, the hepatocytes were quantified for CYP3A4 activity at the time of treatment. Results of the second study show confirm the correlation between with high CYP3A4 activity and sensitivity to hepatotoxic drugs. Our results with primary cultured hepatocytes from multiple donors support the hypothesis that elevated P450 activity may be a risk factor for drug-induced liver injuries.

1. Introduction

Association of marketed drugs with acute liver failures, resulting in deaths or a need for liver transplantation, continues to be a major challenge for the pharmaceutical industry and its regulatory agencies. One puzzling observation is that drugs that are found safe for the vast majority of the human population can cause severe hepatotoxicity in specific individuals. Identification of the human population at-risk to drug-induced liver injuries (DILI) is a vigorous ongoing research effort, which, if successful, would minimize the occurrence of this devastating event. One such effort is the genome-wide association studies (GWAS) through which the major histocompatibility complex (MHC) HLA-B*5701 genotype was found to be associated with DILI due to flucloxacillin [4,5]. However, common genetic variants amongst patients of all DILI drugs for the identification of at-risk populations are yet to be discovered [26,32]. The GWAS findings are consistent with our previous hypothesis that individual differences in sensitivity to hepatotoxic drug may be a result of co-occurrence of multiple, transient,

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Table 1
Hepatotoxicity and key drug metabolizing pathways for the drugs evaluated in this study: acetaminophen, cyclophosphamide, ketoconazole, and tamoxifen.

Drugs	Clinical hepatotoxicity		Metabolism		
	Incidence Rate	References	Major Drug Metabolizing Enzymes	Toxic Metabolites	References
Acetaminophen	>500 acute liver failure per year in United States	[10]	CYP3A4, CYP2E1,CYP1A2, CYP2D6	N-acetyl-p-benzoquinone imine (NAPQI)	[13]
Cyclophosphamide	Severe, acute hepatic failure has been reported	[11,28,29];	CYP2B6, 2C19	Iminocyclophosphamide	[8]
Ketoconazole	4.4% of non-APAP related liver failure in United States from 1990 to 2002	[27]	3A4, FMO (putative)	N-acyl-ketoconazole	[25]
Tamoxifen	Frequently associated with NASH but not with liver failure	[1,7,15]	CYP3A4	alpha-hydroxytamoxifen, alpha,4-dihydroxytamoxifen, and alpha-hydroxy-N-desmethyltamoxifen.	[22]

environmentally-related phenomena and therefore cannot be identified by genome characterization alone [16].

One possible DILI risk factor is drug metabolism capacity which is known to vary greatly among individuals. Drug metabolism is a key determinant of drug toxicity: Toxic drugs can be detoxified into non-toxic metabolites while nontoxic drugs can be bioactivated into toxic metabolites. Drug metabolism occurs mainly in the liver, with the cytochrome P450 dependent monooxygenase (CYP) family of isoforms being the most important drug metabolizing enzymes. CYP3A4 is generally believed to be the most important P450 isoform as it is the most abundant and active, and is known to be responsible for the metabolism of >50% of existing drugs [19]. Furthermore, it is a highly inducible P450 isoform, whereby its activity can be drastically elevated via exposure of an individual to inducers in foods, medications, and pollutants. Both genetic [14] and environmental factors [31] have been attributed to the known vast individual differences in CYP3A4 activity reported in the human population. It is reasonable, therefore, to hypothesize that individuals with exceptionally high CYP3A4 activities will have a higher than normal sensitivity to the hepatotoxicity of drugs that are bioactivated by this isoform.

To test this hypothesis, we evaluated the activities of the 8 major CYP isoforms: CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 in human hepatocytes from 19 individual donors. Based on the results, hepatocytes from multiple donors with CYP3A4 activities encompassing the range of activity observed in the 19 donors were evaluated for their sensitivity to four hepatotoxic drugs that are known to involve CYP3A4 in their bioactivation: Acetaminophen, cyclophosphamide, ketoconazole, and tamoxifen. All four model drugs have been found to be associated with acute liver failures with rare incidences (Table 1).

Our results show that hepatocytes with the highest CYP3A4 activity also had the highest sensitivity to the 4 hepatotoxic drugs, thereby supporting our hypothesis that high P450 activity may be a risk factor for DILI.

2. Materials and methods

2.1. Chemicals

The hepatotoxicants acetaminophen, cyclophosphamide, and tamoxifen were commercially obtained from Sigma–Aldrich (St

Table 2
Donor demographics, post-thaw viability and yield, and P450 isoform-selective substrate metabolism activities (pmol/million cells/min) of the hepatocyte from 19 donors (hepatocyte lots). Individual donor values as well as mean, standard deviation, and correlation of variance (CV) are shown. The hepatocyte lots were organized by CYP3A4 activity, with the highest on top and lowest at the bottom. The six lots chosen for the *in vitro* hepatotoxicity study (underlined and italicized) had CYP3A4 activities representative of the range of the 19 lots, with HH1023 having the highest and HH1033 having the lowest activity. The metabolic pathways evaluated were phenacetin 1-hydroxylation (CYP1A2), bupropion hydroxylation (CYP2B6), paclitaxel 6α–hydroxylation (CYP2C8), diclofenac 4-hydroxylation (CYP2C9), dextromethorphan hydroxylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1), and testosterone 6β-hydroxylation with activity expressed as pmol/min/million hepatocytes. Viability was determined by trypan blue exclusion. C: Caucasian, H: Hispanic; M: Male; F: Female; BMI: Body mass index. The human hepatocyte lots used in the initial study for the evaluation of the relationship between CYP3A4 activity and sensitivity to hepatotoxicants were bolded and underlined.

Lot number	Ethnicity	Gender	Age (years)	BMI	Viability (%)	Yield	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2D6	CYP2E1	CYP3A4
HH1023	***C***	***F***	***21.0***	***23.2***	***89.1***	***4.9***	***237.5***	***507.5***	***2.1***	***74.3***	***112.1***	***229.5***	***843.2***
HH1038	C	M	49.0	29.9	89.0	6.6	96.0	267.0	4.8	115.8	3.2	42.4	733.5
HH1035	C	F	46.0	22.0	89.0	4.4	77.9	10.5	0.9	48.3	10.3	77.6	728.2
HH1006	H	M	35.0	37.0	78.3	4.2	130.0	61.5	5.8	122.5	108.4	105.1	578.0
HH1009	C	F	47.0	27.3	97.4	6.0	311.5	37.9	0.7	42.0	84.6	287.1	569.3
HH1036	***C***	***M***	***55.0***	***26.0***	***94.0***	***7.0***	***37.5***	***39.0***	***1.4***	***74.6***	***6.9***	***49.2***	***512.8***
HH1044	C	M	67.0	21.7	90.0	6.3	115.0	52.9	1.5	55.8	46.2	41.1	477.7
HH1007	***C***	***F***	***26.0***	***26.9***	***94.0***	***5.4***	***140.0***	***143.5***	***3.8***	***80.5***	***177.5***	***240.8***	***398.3***
HH1012	C	M	22.0	25.2	89.7	4.8	669.0	49.1	0.8	60.1	38.6	299.7	395.2
HH1040	C	F	69.0	28.9	94.0	3.9	201.5	96.7	0.5	88.3	16.1	18.1	372.7
HH1047	H	M	44.0	23.5	90.0	5.8	69.6	12.7	1.5	83.7	18.0	47.4	320.3
HH1045	H	M	9.0	19.5	90.0	9.0	51.9	19.5	1.4	90.3	35.5	38.9	311.4
HH1014	C	M	15.0	20.0	72.1	4.6	80.0	118.9	1.5	57.5	70.7	333.1	195.2
HH1031	***H***	***M***	***42.0***	***43.6***	***93.0***	***5.5***	***12.7***	***81.0***	***2.3***	***59.8***	***18.0***	***38.0***	***179.2***
HH1027	***C***	***F***	***59.0***	***32.8***	***90.1***	***5.6***	***28.0***	***66.6***	***0.7***	***58.8***	***11.4***	***120.4***	***126.4***
HH1015	C	M	17.0	23.0	90.4	4.2	95.5	100.0	1.3	21.3	64.1	190.1	107.7
HH1008	C	F	60.0	32.5	93.0	4.4	75.0	33.2	2.2	52.5	80.5	243.7	86.1
HH1029	C	M	51.0	18.0	87.0	4.4	57.0	24.6	0.2	51.0	90.8	285.7	81.9
HH1033	***C***	***F***	***40.0***	***44.9***	***94.0***	***6.2***	***31.1***	***18.3***	***1.3***	***66.0***	***19.4***	***35.9***	***73.9***
Mean			40.7	27.7	89.7	5.4	132.5	91.6	1.8	68.6	53.3	143.4	373.2
Standard Deviation			18.1	7.6	5.8	1.3	150.6	117.6	1.5	24.6	46.8	111.8	241.9
CV			44.3%	27.6%	6.5%	23.1%	113.7%	128.4%	80.7%	35.9%	87.7%	78.0%	64.8%