Genetic Factors and Mechanism underlying Drug-induced Liver Injury

Nottingham Digestive Diseases Biomedical Research Unit

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DILI: Compound to idiosyncrasy

- Context
- Antigenicity
  - Diclofenac
- Immunogenicity
  - Hapten hypothesis: flucloxacillin
  - P-i concept: xymelagatran
  - Altered peptide repertoire model: minocycline
- HLA association with multiple DILI
How useful are animal models?

- 46/75 (61%) candidates developed (2000-10) liver abnormalities in animal toxicology
- 72-79% with hepatotoxicity entered clinical development
- Concordance between animal toxicology and human hepatotoxicity is 55% (17/31)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Therapeutic class</th>
<th>DILI rate (ALT &gt; 3 ULN)</th>
<th>Regulatory outcome</th>
<th>Animal toxicology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flutamide</td>
<td>antiandrogen</td>
<td>1–5%</td>
<td>labeling change</td>
<td>negative</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>NSAID</td>
<td>2.2%</td>
<td>labeling change</td>
<td>positive (high dose in rats)</td>
</tr>
<tr>
<td>Bromfenac</td>
<td>NSAID</td>
<td>2.8%</td>
<td>removal</td>
<td>negative</td>
</tr>
<tr>
<td>Trovaflloxacin</td>
<td>quinolone</td>
<td>2–3%</td>
<td>labeling change</td>
<td>positive (high dose in dogs)</td>
</tr>
<tr>
<td>Zileuton</td>
<td>5-lipoxygenase inhibitor</td>
<td>4.4%</td>
<td>labeling change</td>
<td>negative</td>
</tr>
<tr>
<td>Duloxetine</td>
<td>NERI + SSRI</td>
<td>1%</td>
<td>labeling change</td>
<td>negative</td>
</tr>
<tr>
<td>Lumiracoxib</td>
<td>COX-2 inhibitor</td>
<td>2.6%</td>
<td>removal</td>
<td>negative</td>
</tr>
<tr>
<td>Troglitazone</td>
<td>PPARγ agonist</td>
<td>1.9–4.3%</td>
<td>removal</td>
<td>negative</td>
</tr>
<tr>
<td>Ximelagatran</td>
<td>thrombin inhibitor</td>
<td>6–13%</td>
<td>removal</td>
<td>negative</td>
</tr>
</tbody>
</table>

Drug-induced Liver Diseases: phenotypes

- **DILI**: Liver injury attributed to a medication that isn’t predictable by pharmacological action of the agent
  - Idiosyncratic
  - Acute onset
- **Drug-associated chronic liver disease**
  - Drug associated fatty liver disease
  - Granulomatous hepatitis
  - Secondary sclerosing cholangitis
  - Nodular regenerative hyperplasia
- **Drug-associated tumours**
  - Adenoma, hepatocellular cancer

Risk Factors

**Drug**
- Dose, Hepatic metabolism, Lipophilicity, Biliary excretion

**Host**
- Age, Gender, Ethnicity, Genetic polymorphisms

**Environment**
- Metabolic syndrome, Alcohol intake, Chronic viral infection

- Reactive metabolite
- Covalent adduct
- Immune response

Aithal & Larrey, Mann’s Pharmacovigilance. 2014
Formation of hapten

- Compound to protein reactive molecule

1. Accumulation of reactive metabolites

2. Binding of cellular or circulating proteins to form covalent adducts
Accumulation of reactive metabolites and covalent adducts produces danger signals leading to oxidative stress, mitochondrial swelling, cell death.

Potential for subclinical injury and cytokine production acting as a danger signal for immune mediated reaction.
Diclofenac 150mg started

Diclofenac stopped

- Bilirubin
- ALP
- ALT
- ggt
Reactive metabolite formation & retention

Sinusoidal membrane

Canalicular membrane

DCF

DCF-AG

CYP2C8

5-OH-DCF

UGT2B7

ABCC3

OR: 8.5

ABCC2

OR: 5

DCF-AG

*In vivo* covalent binding

Diclofenac – *Lysine 199*

Hepatocyte
Reactive metabolites
Covalent adducts
Endogenous proteins
Peptide processing + HLA
Danger signals
Cytokines

MHC Class I molecule and DILI

The immune response:
Activation of cytotoxic CD8+ T cells

Activated CD8+ cytotoxic T cell destroys hepatocyte
Patient

- 31 year, male
- Jaundice and itching x 1 week (Aug 2013).
- ‘Jack 3D’ (thought to contain 1,3-Dimethylamylamine) between May and July 2013.
- Flucloxacillin for skin infection 4 weeks prior to the episodes of jaundice.
- Bil: 229 [0-21] µmol/L
- ALT: 2548 [0-45] U/L
- ALP: 144 [40-130] U/L
- Immunoglobulin (Ig) G: 19.1 [5.3-16.5] g/L.
- Serology A, B, C, E, EBV & CMV: -ive
- ANA and LKM Ab: -ive
- Non-actin SMA Ab: +ive
Pregnane X receptor & ADME genes

- CYP3A4 metabolises the drug to 5’-hydroxymethyl flucloxacillin
- 5’-hydroxy methyl metabolite modifies plasma proteins
- PXR: Transcriptional regulator of CYP3A4/5 & transporter genes
- C allele with slow flucloxacillin metabolism and excretion
- Risk of DILI possession of wild type allele (C) = 3.2 (95% CI 1.5-7.0); p=0.003

GWAS: Fucloxacillin ‘DILI’ gene

- 51 cases, 282 gender, ancestry matched controls
- Rs2395029[G] (p=8.4 x 10^{-33})
  - Missense polymorphism in the HCP5 gene and a tag SNP for the HLA-B*5701
- 84% of cases and 5% of controls carried the risk allele G

Cytotoxic T cells in Fluclox-DILI

- FLUX-reacting T cells from patients express chemokine receptors CCR2, CCR4, and CCR9
- Cytotoxic CD3+ CD8+ T cells infiltrate the liver
- FLUX-reacting T cells kill
  - FLUX-presenting autologous Epstein-Barr virus-B lymphoblastoid cell lines
  - HLA-B*57:01-Transduced Hepatocytes

MHC Class II molecule and DILI

The immune response: CD4+ T cells / B Cells

Hapten Pathway

Small chemical compounds bind covalently to endogenous proteins to form hapten–carrier complexes that are antigenic and induce T cell responses.
Patient

- 21 yr student transferred from secondary care on 18 Jan 14
  - 6 days of jaundice and tiredness
  - Coamoxiclav for a boil on the back in Dec 2013
- Bil:138 µmol/l (8.1 mg/dl), ALT:2296 IU/l, ALP:102 IU/l
- PT 16 sec (INR 1.4), creat 65 µmol/l, Bicarb 22 mmol/l, lactate 1.9 mmol/l
  - Hep A,B,C,E, EBV negative
  - ANA, LKM, SMA: negative, Igs: normal
  - Normal ceruloplasmin
  - Paracetamol/ salycylate: levels undetectable
  - Normal US abdomen
- Transjugular liver biopsy (22 Jan 2014)
Follow up

- 29 Jan 2014
  - Bil 479 µmol/l (28.2 mg/dl), ALT: 1931 IU/l
  - PT 27 sec
- Next 72 hours:
  - Progressive rise in PT; Encephalopathy
  - Listed
- 4th Feb 2014:
  - Transplanted
Co-amoxiclav DILI: GWAS

- **HLA-DRB1*1501-DQB1*0602**
- Independent association with HLA-A*0201
- **Ethnic variation**
- **Genotype of Indian patient: HLA-B*61-C*15-DRB1*15:02-DQB1*06:01**

Lucena et al. *Gastroenterology* 2011;141:338-47
MHC Class II molecule

Structure & Molecular Surface of HLA DRB1

- DRB1*15:01 and DRB1*15:02 differ P1
- Residue 86 of DRB1 chain is integral in the formation of P1
  - DRB1*15:01 - V (valine)
  - DRB1*15:02 - G (glycine)
- Neutral in terms of electrostatic potential at P1

Aithal & Donaldson. *Unpublished* 2014
Pharmacological interaction concept

The immune response: CD4+ T cells / B Cells

Drugs or metabolites can bind reversibly to HLA molecules directly

Cytokines

CD4+

B cell

Plasma cell

Antibodies
EpiVax based competitive binding assay

Inhibition of control binding peptide = affinity of ximelagatran to soluble MHC molecules

Pharmacological interaction concept

The immune response: CD4+ T cells / B Cells

Cytokines

CD4+

Altered peptide repertoire

B cell

Plasma cell

Antibodies
Abacavir hypersensitivity syndrome

Abacavir binds non-covalently to HLA-B*57:0, changes the shape and chemistry of the antigen-binding cleft, altering the repertoire of endogenous peptides that subsequently bind.

The ‘altered self’ activates abacavir-specific T-cells, driving polyclonal CD8 T-cell activation and a systemic reaction manifesting as AHS.
Patient

- 18 yr male
- Jaundice
- H/O severe acne
  - Unresponsive to isotretinoin (vit A)
    - Minocycline
- Bil 85 µmol/l
- ALT 500 u/l
- ANA 1:400
- IgG 18.3 (6-15)
- SMA positive
GWAS: Minocycline DILI

- HLA-B*35:02 carrier frequency of 16% in DILI cases vs 0.6% in population controls (Odds Ratio: 29.6, 95% CI: 7.8-89.8; \( p = 2.5 \times 10^{-8} \))
- HLA-DRB1*0301 & *0401 with autoimmune Hepatitis

Urban et al. AASLD 2015
Molecular docking of minocycline in the

- ‘van der Walls contacts’ with HLA-B*35:02 antigen binding cleft

Urban et al. unpublished
GAWS: DILI gene

- HLA-A*33:01 associated with only cholestatic/mixed DILI (n=304): OR 5.2 [3.3-8.2](p=5.2x10^{-13}).
### HLA-A 3301 and DILI

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Hepatic metabolism/ Biliary excretion</th>
<th>Pattern of DILI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terbinafine</td>
<td><img src="image" alt="Terbinafine" /></td>
<td>N-dealkylation; Aldehyde- Glutathione adduct; Canalicular transport</td>
<td>Cholestatic</td>
</tr>
<tr>
<td>Ticlopidine</td>
<td><img src="image" alt="Ticlopidine" /></td>
<td>CYP 450 (2B6)/ carboxoyesterase; Glutathione adduct; Mrp2 transport</td>
<td>Cholestatic</td>
</tr>
<tr>
<td>Finofibrate</td>
<td><img src="image" alt="Finofibrate" /></td>
<td>CYP3A4</td>
<td>Cholestatic</td>
</tr>
<tr>
<td>Sertraline</td>
<td><img src="image" alt="Sertraline" /></td>
<td>CYP 450 (2B6, 2C9, 2C19, 2D6, 3A4); Mitochondrial &amp; ER stress</td>
<td>Hepatocellular</td>
</tr>
</tbody>
</table>

Aithal *et al.* *AASLD* 2015
Making science personal

- EUROHEPATOX Biomed II programme of the European Union

National Institute for Health Research

NDDC
Nottingham Digestive Diseases Centre

Nottingham University Hospitals NHS Trust

DH
Department of Health

SAE Consortium, Ltd.

PRO-EURO DILI REGISTRY