BIOCHEMICAL MECHANISMS OF DRUG TOXICITIES

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TYPES OF ADRs

Hepatic
Cardiac
Skin
Renal
Pulmonary
Neurological
Lupus
Anaphylaxis
Hemolytic anemia
Granulocytopenia
Thrombocytopenia
Aplastic anemia
Vasculitis

SEVERITY OF ADRs

Minor

Severe (SADRs)

- **6.2-6.7% hospitalized patients in USA**
- **over 2 million hospitalized patients**
- **similar findings in Europe and Australia**
- **tens of billions of dollars cost burden**

Wilke, et al., Nature Review-Drug Discovery, 904, 2007

LEADING CAUSES OF DEATH IN USA IN 1994

Heart disease	**743,460**
Cancer	**529,904**
Stroke	**150,108**
SADRs	**106,000**
Pulmonary disease	**101,077**
Accidents	**90,523**
Pneumonia	**75,719**
Diabetes	**53,894**

Lazarou et al., JAMA, 279, 1208 (1998)

DRUGS WITHDRAWN IN USA

Azaribine, psoriasis, blood clots, 1976
Ticrynafen, blood pressure, liver injury, 1980
Benoxaprofen, NSAID, liver toxicity, 1982
Zomepirac, NSAID, anaphylaxis, 1983
Nomifensine, anti-depressant, hemolytic anemia, 1986
Suprofen, NSAID, kidney failure, 1987
Temafloxacin, antibiotic, kidney failure, 1992
Fenfluramine, appetite suppression, heart valve disease, 1997
Terfenadine, anti-histamine, fatal arrhythmia, 1998
Bromfenac, NSAID, liver injury, 1998
Mibefradil, blood pressure, muscle damage and fatal arrhythmia, 1998

DRUG WITHDRAWN IN USA

Grepafloxacin, antibiotic, fatal arrhythmia, 1999
Astemizole, antihistamine, fatal arrhythmia, 1999
Cisapride, heartburn, fatal arrhythmia, 2000
Troglitazone, diabetes, liver toxicity, 2000
Cerivastatin, cholesterol reduction, muscle damage leading to kidney failure, 2001
Etretinate, psoriasis, birth defects, 1999
Levomethadyl, opiate dependence, fatal arrhythmia, 2008
Rofecoxib, NSAID, heart attack, stroke, 2004
Valdecoxib, NSAID, skin disease, 2005
Pemoline, ADHD, liver toxicity, 2005

TYPE A ADRs

80% of ADRs
Relatively frequent and often predictable
Excessive or diminished pharmacologic effects
Drug-drug interactions and polymorphisms
Mild to severe ADRs
Often uncovered preclinically

Endres, et al., European Journal of Pharmaceutical Sciences, 27, 501 (2006)

EXAMPLES OF TYPE A ADRS

Drowsiness from antihistamines

Hypotension from antihypertensive therapy

Excess bleeding from warfarin

Prolonged neuromuscular blockade by serum choline esterase deficiency

Acetaminophen

TYPE B ADRs

20% of ADRs

Rare, unpredictable, and highly host-dependent
Mild to severe ADRs

Rarely uncovered preclinically in animals or in clinical trials

Mechanisms often unknown but may be due to:

- **Allergic Reactions**
- **Rare Polymorphisms**
- **Imbalance in Cellular Homeostasis**

HAPTEN HYPOTHESIS AND DRUG-INDUCED ALLERGIC REACTIONS

Graphic illustration of drug or metabolite reactive with body proteins that then trigger B- and T-cell immune responses.

Graphic illustration of drug-protein conjugate presented as antigen to dendritic cells.

MECHANISMS OF DRUG-INDUCED IMMUNE-MEDIATED BLOOD DYSCRASIAS

Graphic illustration of drug-antibody complex on cell surface leading to complement activation and cell lysis.

CUTANEOUS DRUG REACTIONS

95% are self-limiting rashes

SJS and TEN can be life-threatening with blisters, skin detachment, and mucosa involvement

Most appear to be immune-mediated by drug-specific IgE antibodies while many others by $CD4^+$ and $CD8^+$ T cells

Roychowdhury and Svensson, AAPS J., 7, E 434 (2005)

MACULO-PAPULAR EXANTHEM AND TOXIC EPIDERMAL NECROLYSIS

Photos of two individuals suffering from maculo-papular exanthem and toxic epidermal necrolysis.

T CELL REACTIVITY TO DRUGS CAUSING CUTANEOUS ADRS

Lidocaine

Sulfonamides

β-Lactam antibiotics

Phenytoin

Carbamazepine

Lebrec et al., Cell Biology and Toxicology, 15, 57 (1999); Naisbitt, et al., Expert Opin. Drug Saf., 6, 109 (2007); Posadas and Pichler, Clin. Experimental Allergy, 37, 989 (2007)

Graphic illustration of drug bioactivation, hapten conjugate processing and T-cell immune response in the skin.

HLA-B*1502 ASSOCIATED WITH CBZ-INDUCED SJS/TEN

Seen in south-east Asians but not in Caucasians

98.3% (59/60) CBZ-SJS/TEN positive

4.2% (6/144) CBZ-tolerant positive

High sensitivity/specificity of this test can be used to screen patients receiving CBZ

Chung, et al., Curr. Opin. Allergy Clin. Immunol., 7, 317 (2007)

IgE-MEDIATED ANAPHYLACTIC DRUG REACTIONS

Alcuronium
Cephalosporins
Penicillins
Protamine
Streptokinase
Sulfamethoxazole
Suxamethonium
Thiopentone
Trimethoprine
Tubocurarine

Park et al., Chem. Res. Toxicol., 11, 969 (1998);Thong and Chan, Ann. Allergy Asthma Immunol., 92, 619 (2004)

MECHANISM OF DRUG-INDUCED ANAPHYLAXIS

lgE-mediated mast cell release of histamine, leukotrienes and cytokines.

Graphic illustration

Airway smooth muscle contraction leading to bronchospam

Increase permeability of blood vessels and mucous gland secretion

Inflammation (eosinophils and neutrophils)

Respiratory, gastrointestional, cutaneous, and cardiovascular systems can be involved

DRUG-INDUCED LIVER DISEASE IS A MAJOR HEALTH PROBLEM

It is a major cause of acute liver failure and a major safety reason for:

- **Stopping preclinical development of drugs**
- **Terminating clinical trials of drugs**
- **Withdrawing drugs postmarketing**

F. Ballet, J. Hepatol., 26 (Suppl. 2), 26 (1997)

DRUGS WITHDRAWN / NOT APPROVED DUE TO LIVER DISEASE

Iproniazid	**1956**
Ibufenac (Europe)	**1975**
Ticrynafen	**1980**
Benoxaprofen	**1982**
Perhexilene (France)	**1985**
Dilevalol (Portugal and Ireland)	**1990**
Bromfenac	**1998**
Troglitazone	**2000**
Nefazodone (Serzone)	**2003**
Ximelagatran (Exanta)	**2004**

ACETAMINOPHEN LIVER INJURY

Chemical structures of acetaminophen metabolites.

MITOCHONDRIAL DAMAGE IN AILI

Graphic illustration of liver mitochondrial injury due to NAPQI, a toxic metabolite.

DRUGS CAUSING DILI ASSOCIATED WITH MITOCHONDRIAL INJURY

Troglitazone
Diclofenac
Nimesulide
Mefenamic acid
Tolcapone
Valproic acid
Leflunomide
Amiodarone
Trovafloxacin
Simvastatin
Perhexiline
Isoniazid
Dantrolene
Sulindac
Lamivudine
Stavudine
Fialuridine

U.A Boelsterli and P.L.K. Lim., Toxicol. Appl. Pharmacol., 220, 92 (2007)

FIALURIDINE-INDUCED MITOCHONDRIAL INJURY IN PATIENTS

FIAU is a uridine analog developed for hepatitis B treatment

Administration to 15 patients resulted in 7 developing severe mitochondrial liver damage with 5 dying and 2 receiving liver transplant

Toxicity was not predicted from rodent studies

MECHANISM OF FIAU LIVER INJURY

Toxicity of FIAU is apparently due to FIAU-TP which inhibits mitochondrial DNA polymerase-γ and DNA synthesis

Humans and not rodents have human nucleoside transporter 1 (hENT1) in the mitochondrial membrane

E.W. Lee, et al., J.Biol.Chem., 281, 16700 (2006)

INNATE IMMUNE CELL INJURY CAN FOLLOW INITIAL INTRINSIC DILI

DAMPs: HMGB-1, MIF, HSPs

Protoxicant Factors: IFN-γ, osteopontin, IL-6, ROI, RNI

Protective Factors: IL-4, IL-6, IL-10, IL-13, COX-2

Cells: Kupffer cells, PMNs, NK, NKT cells and hepatocytes

M.E. Bianchi, J. Leukoc. Biol., 81, 1 (2007); D.J. Antoine et al., Expert Opin. Drug Metab.Toxicol, 4, 1415 (2008)

INFLAMMATORY CELL INVOLVEMENT IN AILI IN A IL-10 KNOCKOUT MOUSE

Electron microscopy of liver tissue injury.

PAMPS CAN ACTIVATE THE INNATE IMMUNE SYSTEM

TLR1/2 and TLR2/6 activated by bacterial triacylated and diacylated lipopeptides, respectively

TLR4 activated by LPS, several HSPs, heparan sulfate products, hyaluronic acid fragments

TLR5 activated by bacterial flagellin

TLR 3 activated by viral dsRNA

TLR7 and 8 activated by viral ssRNA

TLR9 activated by bacterial unmethylated CpG DNA

E. Seki and D.A. Brenner, Hepatology, 48, 322 (2008)

POTENTIAL ROLE OF GUT-DERIVED LPS ENDOTOXIN IN DILD

Rat and mouse models of DILD have been produced by LPS + drug treatments

Diclofenac, chlorpromazine, trovafloxacin, and ranitidine

LPS activates TLR4 which can lead to activation of monocytes, macrophages, dendritic cells, mast cells and other cells.

P. J. Shaw et al., Toxicol. Sci. 107, 270 (2009)

HALOTHANE-INDUCED ALLERGIC HEPATITIS

Graph of halothane P450-mediated metabolism.

HALOTHANE HEPATITIS PATIENTS' SERUM ANTIBODIES (% REACTIVITY)

ANTIGEN	TFA-PROTEIN	NATIVE-PROTEIN
PDI	10	5
PDI isoform	55	25
Carboxylesterase	13	5
Calreticulin	5	3
ERP72	30	25
GRP94	65	28
CYP2E1		45

OTHER HALOTHANE DERIVATIVES

Chemical structures of halothane, isoflurane, and desflurane, all substrates of P450 enzymes.

ANTIBODIES ASSOCIATED WITH OTHER DRUGS CAUSING HEPATITIS

Drug	Antigen
Tienilic acid	CYP2C9
Dihydralazine	CYP1A2
Ethanol	CYP2E1, CYP3A4, CYP2E1-hydroxy-ethyl radical

T CELL REACTIVITY ASSOCIATED WITH DRUGS CAUSING ALLERGIC HEPATITIS

Cotrimoxazole
Erythromycin
Ketoconazole
Ampicillin
Allopurinol
Ibuprofen
Captopril
α-Methyldopa
Enalapril

Chlorpromazine
Amineptine
Dothiepine
Phenytoin
Carbamazepine
Tamoxifen
Glibenclamide
Lovastatin
Propylthiouracil

Gut, 41, 534 (1997)

HEPATOTOXIC DOSE OF APAP

DEPLETES LYMPHOCYTES WITHIN 24 HOURS

Bar charts showing Splenocytes in the Spleen, Thymus, and Hepatic Lympth Nodes with control, 80 mg/kg and 300 mg/kg

M.J. Masson, et al., Chem. Res. Toxicol., 20, 20 (2007)

DNCB AS A MODEL DRUG ALLERGEN

Chemical structures of dinitrochlorobenzene and metabolites.

PAINTING DNCB ON SKIN CAUSES DTH IMMUNE REACTION

TOLERANCE PROTOCOL

Graphic illustration desensitization process.

C. Ju, et al., Chem. Res. Toxicol., 16, 1514 (2003).

Summary

Drug-drug interactions are the major cause of ADRs, but are often predictable. Polymorphisms can also play a role.

Many SADRs are rare, highly host-dependent and difficult to predict. Multiple genetic and environ-mental factors may have a role as well as the innate and adaptive immune systems.

Designing drugs that will not be metabolized to reactive metabolites may eliminate many SADRs

Newer preclinical screening tests may also prevent many SADRs