Mechanisms of Idiosyncratic Drug Reactions and Their Implications for Risk Prediction

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Idiosyncratic Drug Reactions (IDRs)
(The definition depends on who is doing the defining)

• Do not occur in most patients at any dose.
• Do not involve the pharmacological effects of the drug, e.g. warfarin-induced skin necrosis.
• Other terms are often used, e.g. hypersensitivity reactions, allergic reactions, type B (bizarre) reactions.
• No current definition is perfect and if we understood the mechanism the term idiosyncratic would probably become obsolete.
Characteristics of IDR

• Unpredictable
• Delay in onset, which varies with the type of IDR and even the drug.
• On reexposure, typically the IDR occurs immediately but there are many exceptions.
• The same drug can often cause more than one type of IDR.
• There may be no increase in incidence with increasing dose within the therapeutic range, but IDRs are not dose independent!
**IDRs and Drug Development**

- 20 years ago the major reason for drug candidate failure was pharmacokinetic; now the major reasons are lack of efficacy and toxicity.
- Idiosyncratic reactions represent the largest problem for safety because of their unpredictable nature, and therefore they introduce a significant degree of risk into the process of drug development.
- An understanding of the basic mechanisms of idiosyncratic drug reactions is essential for predicting risk.
Relationship Between Reactive Metabolites and Idiosyncratic Drug Reactions (IDRs)

- Most drugs that are associated with a relatively high incidence of IDRs form a significant amount of reactive metabolite.
- Analogs that form less reactive metabolite are safer, e.g. isoflurane vs halothane.
- However, some drugs that form a lot of reactive metabolite do not cause a significant incidence of IDRs, and some drugs that cause IDRs do not appear to form reactive metabolites (caveat: our measurements of reactive metabolites are inaccurate).
- The fact that the liver is the major site of drug metabolism is presumably the reason it is a major site of IDRs.
- The evidence for involvement of reactive metabolite involvement is circumstantial, and it is difficult to prove that a specific reactive metabolite is responsible for a specific IDR.

Comparison of Covalent Binding to Neutrophils of 3 Drugs that Cause Agranulocytosis
What is the Role of Reactive Metabolites in the Mechanism of IDRs?

- Hapten Hypothesis
- Danger Hypothesis
- P-I Hypothesis
- Mitochondrial Toxicity
- Inflammmagen Hypothesis
- Inhibition of DNA methylation or other epigenetic effects
- Reactivation of Herpes Virus
Hapten Hypothesis

- Some IDRs such as penicillin-induced allergic reactions definitely involve this mechanism.
- Some drugs such as halothane, tienilic acid, diclofenac, and dihydralazine clearly induce an immune response against drug-modified proteins and/or against the native protein (autoantibodies) that was modified by the reactive metabolite.
- It has not been demonstrated that this immune response mediates the IDR but it is likely that it does.
- Other factors such as danger/unfolded protein response may also be involved in these cases.
Danger Hypothesis

- This an attractive hypothesis because something has to upregulate signal 2 in order to induce an immune response.
- The ability to cause cell damage (danger signal) could differentiate which reactive metabolites are likely to cause IDR.
- We have found changes in gene expression consistent with the danger hypothesis, but the changes vary with the drug so there is no obvious basis for a universal biomarker and it is very difficult to definitively test the hypothesis.
Hapten and Danger Hypotheses

Drug → Reactive metabolites → Modified protein

Without Signal 2 → Tolerance

With Signal 2

Signal 1 → Helper T cell

Signal 2 → Helper T cell

APC → Helper T cell

B7 → CD28

CD8+T

B cell

Immune response
P-I Hypothesis

• Involves reversible binding of the parent drug to MHC/T cell receptor complex – no reactive metabolite is involved.
• It is based on observations with cloned T cells and the assumption that what these T cells respond to is what induced the immune response and we have shown this to be false.
• Much of the data involves sulfamethoxazole, which is an aromatic amine, and virtually all primary aromatic amines cause IDR\(s\) presumably because they form reactive metabolites.
Mitochondrial Toxicity

- Drugs can certainly cause toxicity, in particular hepatotoxicity, through effects on mitochondria, e.g. acetaminophen, valproic acid, and NRTIs.
- Most of the data suggesting this is a major mechanism of hepatotoxicity are *in vitro*.
- The mitochondrial SOD heterozygote model is very attractive but others have not been able to repeat the results.
- I doubt that this is the primary mechanism of idiosyncratic drug-induced liver injury in the absence of steatosis/lactic acidosis; however, mitochondrial stress could be a danger signal.
Inhibition of DNA Methylation

• Richardson has compelling data to suggest that some drug-induced autoimmunity (procainamide & hydralazine) involves inhibition of DNA methylation.
• Some drugs such as pyrazinamide and allopurinol that are associated with a high incidence of IDR's do not appear to form reactive metabolites.
• Pyrazinamide was reported to inhibit DNA methylation and cause delayed liver damage in rats, but we have not been able to reproduce these results.
Inflammmagen Model

- The combination of ranitidine and LPS causes liver injury in rats that does not occur with either agent alone.
- However, ranitidine is a safe over-the-counter drug.
- The assay is negative for isoniazid, the drug associated with the highest risk of idiosyncratic DILI.
- The biological response to LPS is rapidly down-regulated so its action is limited to a one time dose and drug-induced liver failure is caused by sustained liver damage.
- Most important, the characteristics are wrong:
  - The toxicity is acute rather delayed – time to onset is not random
  - The histology is dominated by neutrophils rather than lymphocytes
- We have used co-treatment with LPS and Poly-IC to try to stimulate the immune system but it did not lead to an animal model.
Importance of Animal Models

- Virtually impossible to do prospective studies in humans.
- Animal models are essential to rigorously test hypotheses and to study the detailed series of steps involved in human diseases.
- To be useful, an animal model must involve essentially the same mechanism as the IDR in humans.
- Therefore, it is essential to continuously test any animal model against the idiosyncratic reaction in humans.
- Animals are not people, but idiosyncratic reactions vary among humans, and a specific animal may be a better model for a specific human than another human (J. Gillette).
Example of a Useful Animal Model: Nevirapine-Induced Skin Rash in Rats

- Nevirapine causes a skin rash in 8-16% of patients and can also cause severe liver toxicity.
- Nevirapine also causes a skin rash in rats and an increase in ALT in mice.
## Comparison of Rash in Humans and Rats

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Humans</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash</td>
<td>mild morbilliform to TEN</td>
<td>mild to severe but no blisters</td>
</tr>
<tr>
<td>Plasma levels</td>
<td>1-10 µg/ml</td>
<td>20-40 µg/ml</td>
</tr>
<tr>
<td>Time to onset</td>
<td>1-3 weeks</td>
<td>2-3 weeks</td>
</tr>
<tr>
<td>Dose response</td>
<td>incidence increases with dose</td>
<td>incidence increases with dose</td>
</tr>
<tr>
<td>Sex dependence</td>
<td>incidence greater in women</td>
<td>incidence greater in females but probably metabolic difference</td>
</tr>
<tr>
<td>Low dose pretreatment</td>
<td>decreases incidence</td>
<td>prevents rash</td>
</tr>
<tr>
<td>Rechallenge</td>
<td>immediate onset and more severe</td>
<td>decreased time to onset and decreased dose required</td>
</tr>
<tr>
<td>Histology</td>
<td>little data, lymphocytic infiltrate</td>
<td>T cells and macrophages</td>
</tr>
<tr>
<td>T cell dependence</td>
<td>incidence low if CD4+ count low</td>
<td>depletion of CD4+ T cells is protective.</td>
</tr>
<tr>
<td>In vitro lymphocyte response to NVP</td>
<td>produce IFN-γ</td>
<td>produce IFN-γ</td>
</tr>
</tbody>
</table>
Nevirapine-Induced Skin Rash is Immune-Mediated

- Mononuclear cells in skin of rats with rash (CD4 & CD8 T cell, macrophages)
- Syndrome occurs earlier (red ears ~8 h and lesions within days) and is more severe on reexposure
- Sensitivity can be transferred to naïve rat with spleen cells or just CD4 T cells.
- Rash not prevented by depletion of CD8 T cells, but depletion of CD4 T cells is partially protective.
- Some of the CD8 T cells are Fox P3+, i.e T regs.
Is the rash caused by the parent drug or a reactive metabolite?
Putative Reactive Metabolite

\[ \text{Putative Reactive Metabolite} \]

\[ \text{P450} \rightarrow \text{sulfotransferase} \]

\[ + \text{SO}_4^{2-} \]
# 12-Hydroxynevirapine Induces Rash

<table>
<thead>
<tr>
<th>Dose</th>
<th>NVP</th>
<th>12-OH NVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg/kg/day</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50 mg/kg/day</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>75 mg/kg/day</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Changes induced by 12-OH NVP in skin at 6 hr (> 400 significant changes)

<table>
<thead>
<tr>
<th>Gene Title</th>
<th>p-value</th>
<th>Fold-Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>tripartite motif-containing 63</td>
<td>0.009</td>
<td>18 Ubiquitin ligase, autophagy</td>
</tr>
<tr>
<td>S100 calcium binding protein A7A</td>
<td>0.008</td>
<td>7 psoriasis, binds to RAGE, activates macrophages, alarmin</td>
</tr>
<tr>
<td>interleukin 22 receptor</td>
<td>0.0008</td>
<td>4 IL-22 but not IL-22r mRNA elevated in psoriasis</td>
</tr>
<tr>
<td>pyruvate dehydrogenase kinase 4</td>
<td>0.005</td>
<td>3.6 induced in liver by hepatotoxins, mitochondrial enzyme</td>
</tr>
<tr>
<td>3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2</td>
<td>0.001</td>
<td>3.4 mitochondrial enzyme, increased in stress</td>
</tr>
<tr>
<td>death associated protein kinase 1</td>
<td>0.0005</td>
<td>3.4 increases apoptosis, induces autophagy, induced by burns</td>
</tr>
<tr>
<td>lipin 1</td>
<td>0.004</td>
<td>3.4 Induced by hypoxia and stress</td>
</tr>
<tr>
<td>uncoupling protein 3</td>
<td>0.004</td>
<td>3.3 mitochondrial protein, decreases ROS</td>
</tr>
<tr>
<td>DnaJ (Hsp40)</td>
<td>0.02</td>
<td>3.2 “stress regulator”</td>
</tr>
<tr>
<td>FK506 binding protein 5</td>
<td>0.009</td>
<td>3.0 forms complex with Hsp70/90, increased in CD34 cells in RA</td>
</tr>
<tr>
<td>Kruppel-like factor 15</td>
<td>0.0005</td>
<td>3.0 oxidative stress, apoptosis</td>
</tr>
<tr>
<td>F-box protein 32</td>
<td>3 E−06</td>
<td>2.8 ubiquitin proteosome pathway, involved in apoptosis</td>
</tr>
</tbody>
</table>
Use of Deuterium Isotope Effect to Specifically Inhibit Reactive Metabolite Formation
Comparison of Nevirapine and Deuterated Nevirapine Levels
(75 mg/kg/day suspension s.c.)
The free radical intermediate partitions between oxygen rebound to form the 12-hydroxy metabolite and loss of another hydrogen to directly form the quinone methide and there is less inhibition with the deuterated analog which leads to more rapid metabolism.
Nevirapine Binds to Hepatic Proteins in vivo and CYP3A1 in vitro
Using ABT to Inhibit P450 leads to Similar Levels of NEV and DNEV
Incidence of Skin Rash in F BN Rats, with DNVP/NVP+ABT treatment (n=5)
Does the specificity of T cells indicate what initiated an immune response?

- The observation that T cells from patients with an IDR to sulfamethoxazole proliferated when incubated with sulfamethoxazole in the absence of metabolism is what led to the p-i hypothesis.
- This seemed improbable because sulfamethoxazole is an aromatic amine which readily forms reactive metabolites.
- The unstated assumption is that what the T cells respond to is what initiated the immune response.
Analogs Tested in LTT

- nevirapine
- 12-hydroxynevirapine may form reactive sulfate but probably not in Tcells
- 4-chloronevirapine looks like nevirapine but it can not form reactive metabolite
- 12-chloronevirapine is even more reactive than sulfate
Specificity of T Cells

Antigen Dependent IFNg Secretion

<table>
<thead>
<tr>
<th>Concentration (microgram/ml)</th>
<th>IFNg (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

- CON/NVP
- NVP/NVP
- CON/12OH
- NVP/12OH
- CON/4Cl
- NVP/4Cl
- CON/12Cl
- NVP/12Cl
Specificity of T cells when the rash was induced by 12-hydroxynevirapine
There is a Disconnect Between What Causes a Rash and LTT
The Basis for the PI Hypothesis is False

nevirapine causes rash, + LTT
12-hydroxynevirapine causes rash, - LTT
4-chloronevirapine no rash, + LTT
12-chloronevirapine - LTT, toxicity?
D-penicillamine Induced Autoimmunity in BN Rats

- Anti-nuclear antibodies
- Skin rash
- Immune complexes in the kidney
- Swollen, red arthritic limbs
- Weight loss
- Hepatic Necrosis
- No decrease in delay on rechallenge

20 mg/day for ~3 weeks

~50-80% incidence
Activation of Macrophages by Penicillamine

- One type of interaction between APCs and T cells involves a reversible imine bond formed by an amine on the T cell and an aldehyde on the APC.

- Formation of an irreversible adduct between penicillamine and the same aldehyde group may lead to activation of APCs and autoimmunity.
Protein targets of ARP

ARP binding membrane proteins

2.2 mM

ARP+ ARP- MPF Ladder

Daltons
211,806
121,020
100,216
54,395
38,708
29,806
20,040
7,331
There is a “New” Kind of T Helper Cell - Th17

Cytokine Profiles in Penicillamine-Treated Rats

**IL-6**

**IL-17**

**IL-6**

**IL-22**
Flow Cytometry (Penicillamine-Treated Rats)

P<0.01

n=12.
What is the mechanism of most IDR s in people?

- Clearly drug-induced autoimmunity is immune-mediated.
- There is ample evidence that drug rashes are immune-mediated.
- Most idiosyncratic drug-induced hematotoxicity appears to be immune-mediated.
- The major question arises with idiosyncratic liver toxicity – immune-mediated vs metabolic idiosyncrasy, e.g. isoniazid-induced hepatotoxicity.
Interindividual Differences in IDR

- One patient treated with nevirapine may develop a skin rash while another may develop DILI. Even the skin rash may be significantly different in one patient than another.
- Some patients treated with minocycline develop DILI with characteristics typical of idiosyncratic liver toxicity such as a time to onset of a couple months, while in others the delay is more than one year and there is definite evidence of autoimmune hepatitis.
- Even though abacavir-induced hypersensitivity and flucloxacillin-induced DILI only occur in patients who are HLA B5701, only about 1/1000 of these patients develop and IDR when treated with either of these drugs.
Drugs can cause a variety of IDRs that often includes autoimmunity

- Isoniazid
- Minocycline
- α-Methyldopa
- Hydralazine
- Nitrofurantoin
- Propylthiouracil
- Methimazole
- Aminoglutethimide

- Phenylbutazone
- Benoxaprofen
- Phenytoin
- Sulfonamides
- Carbamazepine
- \( p \)-Aminosalicylic acid
- Dapsone
- Phenothiazones
T Cell Receptor Repertoire

- T cell receptors (TCR) are formed by random gene recombination and so they are different in every individual, even in identical twins.
- If the TCR with the highest affinity recognizes a drug-modified liver protein it could lead to liver toxicity while if it recognizes a skin protein it may lead to a skin rash.
- If the dominant epitope is mostly native protein it may lead to an autoimmune reaction.
- Even when a specific idiosyncratic reaction only occurs in patients with a specific MHC gene, only about 1/1000 patients with that MHC will have an IDR to that drug.
- Patients with the right MHC for an IDR may not have the right TCR.
Drug → Reactive Metabolite (Hapten) → Protein → Haptenized Protein → Classical anti-drug adaptive immune response

Mixed but mostly typical adaptive immune response

Autoimmune response

T cells with various T cell receptor specificity—Some against the hapten and some against the protein
Why is it difficult to develop animal models of IDRs?

• If, as I am now convinced is true, almost all IDRs are immune-mediated, it may require a specific T cell receptor and MHC molecule.

• More important, it requires overcoming immune tolerance.
Why is it difficult to overcome immune tolerance?

- Unlike the immune response to a pathogen, most of the immunogen produced by covalent binding of a drug to an endogenous protein is a “self molecule”.
- This is similar to the problem faced by oncologists who try to get the immune system to destroy cancer cells that express antigens not found on normal cells. They have not been able to achieve sustained immune responses because of immune tolerance induced by the self antigens.
ALT of Female Cbl-b (-/-) Mice (n = 3)

Weeks

Control
AQ

U/L

0 200

1 100

2 50

3 0
Changes in gene expression induced by amodiaquine in the liver at 7 days.

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Title</th>
<th>Fold Change</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytokine/chemokine and receptor</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CCL5</td>
<td>Chemokine ligand 5</td>
<td>3.4572</td>
<td>0.000156</td>
</tr>
<tr>
<td>CCL12</td>
<td>Chemokine ligand 12</td>
<td>3.3057</td>
<td>0.000129</td>
</tr>
<tr>
<td>CCL6</td>
<td>Chemokine ligand 6</td>
<td>2.5747</td>
<td>0.000009</td>
</tr>
<tr>
<td>CCL2</td>
<td>Chemokine ligand 2</td>
<td>2.1708</td>
<td>0.002232</td>
</tr>
<tr>
<td>Cxcl9</td>
<td>Chemokine ligand 9</td>
<td>1.8928</td>
<td>0.007123</td>
</tr>
<tr>
<td>Ccl17</td>
<td>Chemokine ligand 17</td>
<td>-2.0855</td>
<td>0.002556</td>
</tr>
<tr>
<td>IL4</td>
<td>Interleukin 4</td>
<td>2.9732</td>
<td>0.006743</td>
</tr>
<tr>
<td>IL10</td>
<td>Interleukin 10</td>
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<td>0.01158</td>
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<tr>
<td>IL1b</td>
<td>Interleukin 10</td>
<td>2.3991</td>
<td>0.00015</td>
</tr>
<tr>
<td>IL12b</td>
<td>Interleukin 12b</td>
<td>2.0734</td>
<td>0.007318</td>
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<tr>
<td>IL18</td>
<td>Interleukin 18</td>
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<td>0.000913</td>
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<tr>
<td>IL6</td>
<td>Interleukin 6</td>
<td>1.7184</td>
<td>0.049982</td>
</tr>
<tr>
<td>IL8rb</td>
<td>Interleukin 8 receptor beta</td>
<td>2.3634</td>
<td>0.00382</td>
</tr>
<tr>
<td>IL22ra2</td>
<td>Interleukin 22 receptor alpha 2</td>
<td>1.5075</td>
<td>0.018247</td>
</tr>
<tr>
<td>INF-γ</td>
<td>Interferon gamma</td>
<td>3.0805</td>
<td>0.010875</td>
</tr>
<tr>
<td>Tnfsf14</td>
<td>Tumor necrosis factor superfamily member 14</td>
<td>3.076</td>
<td>0.004718</td>
</tr>
<tr>
<td>Faslg</td>
<td>Fas ligand</td>
<td>2.6639</td>
<td>0.00116</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
<td>1.8115</td>
<td>0.01760</td>
</tr>
</tbody>
</table>
Patient Treated with Isoniazid

IL-22

CD4

IL-17

AST  ALT
21    34

AST  ALT
31    44*
Conclusions (implications of reactive metabolites)

- Reactive metabolites appear to be responsible for many, but not all, IDRs.
- Some types of reactive metabolites are more “dangerous” than others. Dose is also important.
- It is difficult to produce definitive evidence for what chemical species and by what mechanism an IDR is induced; valid animal models represent a powerful tool for such studies but are difficult to establish.
- Although screening for reactive metabolites will not guarantee safe drugs, the formation of reactive metabolites by a drug candidate is a significant liability and dose is also important.
Conclusions (likely mechanism)

• With the exception of liver toxicity, the evidence is compelling that IDRs are immune-mediated, and it is my opinion that idiosyncratic liver toxicity is also immune-mediated, some with an autoimmune component.

• Different people can have different idiosyncratic reactions to the same drug, which may depend on the individual T cell receptor repertoire of the patient.

• It appears that most people respond to drugs that cause IDRs with immune tolerance.
Conclusions (implications for drug development)

- Drugs cause changes that may represent biomarkers that make it possible to predict the risk that a drug candidate will cause IDRss; however, it looks like the biomarkers will be different for different drugs.
- Until we have a better understanding of exactly how drugs cause IDRss (induce an immune response) they will probably remain unpredictable.