How Reactive Metabolites Induce an Immune Response that Sometimes Leads to an Idiosyncratic Drug Reaction

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It is very difficult to study the mechanisms of idiosyncratic drug reactions (IDRs).

- Given the idiosyncratic nature of IDRs, it is virtually impossible to prospectively study them in humans.
- They are also idiosyncratic in animals, and therefore until recently there were no practical animal models that had characteristics similar to IDRs in humans. In general, previous animal models involved acute toxicity in animals given high doses of drug.
- *In vitro* studies simply cannot match the complexity of IDRs.
- Therefore, despite many hypotheses, almost nothing is known with certainty about the mechanisms of IDRs.
Evidence that IDRs are Immune Mediated

- General characteristics such as the idiosyncratic nature, and delay in onset but rapid onset with rechallenge are typical of an immune mediated reaction.
- Histology is strongly suggestive of an immune mediated reaction.
- HLA associations: Several IDRs are associated with a specific HLA. This has not been tested for most IDRs because it is difficult to obtain DNA from a significant number of patients with a history of an IDR to a specific drug, and if the HLA is common it may require a large number of samples. It would not be surprising if there were few strong HLA associations because many reactive metabolites modify thousands of proteins.
- Lymphocyte transformation tests. This provides evidence for an immune mechanism, but is often falsely negative, probably because the system does not generate metabolites.
- Presence of anti-drug antibodies. This requires the availability of a suitable antigen.
- There is little debate except for idiosyncratic drug-induced liver injury (IDILI).

Evidence for Involvement of Reactive Metabolites

- Some functional groups called “structural alerts” that readily form reactive metabolites are associated with a high incidence of IDRs. These include aromatic amines, aromatic nitro groups, thiono sulfur, thiophenes, hydrazines, etc.
- Some IDRs are associated with antibodies against drug-modified proteins.
- However, some drugs that are associated with a high incidence of IDRs do not appear to form reactive metabolites.
- We do not know what protein targets are important.
- Many drugs have the potential to form several reactive metabolites.
- It is exceedingly difficult to prove the involvement of a specific reactive metabolite without a valid animal model where controlled experiments can be performed including modification of metabolic pathways.
For example, carbamazepine has the potential to form many reactive metabolites.

That raises the question: how do reactive metabolites induce an immune response?

**Hapten Hypothesis:** Reactive metabolites covalently bind to proteins forming neoantigens. Recognition of foreign peptides by specific T cells produces *signal 1*.

**Danger Hypothesis:** Foreign proteins do not induce a significant immune response unless they can activate antigen presenting cells (APCs). (“Adjuvants are the immunologists’ dirty little secret.”) This upregulates costimulatory molecules on APCs that are necessary for activation of T cells (*signal 2*). Cell damage caused by a reactive metabolite could lead to the release of danger associated molecular pattern molecules (DAMPs) that can produce signal 2.
Testing Hypotheses

- As mentioned, controlled experiments cannot be performed in humans, and in vitro tests cannot mimic the complexity of the immune system.
- Animal models are the only practical way to rigorously test hypotheses.
- However, the animal model must have essentially the same mechanism as the IDR in humans; therefore, the characteristics should be the same.
The Nevirapine-Induced Skin Rash Model

- Nevirapine causes a skin rash in ~10% of treated patients, and some of these rashes are life-threatening (it can also cause liver failure.)
- We have developed a unique animal model of nevirapine-induced skin rash in Brown Norway rats. It is immune-mediated and has characteristics very similar to the rash in humans.
- It is the only animal model of an IDR similar to the IDR in humans that occurs without manipulation of the immune system, and it was discovered by accident!

Comparison of Rash in Humans and Rats

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Humans</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Level</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>Sex dependence</td>
<td>Incidence greater in women</td>
<td>Hard to produce in male rats</td>
</tr>
<tr>
<td>Histology</td>
<td>data sparse; lymphocytic infiltrate</td>
<td>T cells and macrophages</td>
</tr>
<tr>
<td>T cell dependence</td>
<td>Incidence low in patients with low CD4 T cell count</td>
<td>Depletion of CD4 T cells is protective</td>
</tr>
<tr>
<td>In vitro lymphocyte response to nevirapine</td>
<td>Produce IFN-g</td>
<td>Produce IFN-g</td>
</tr>
</tbody>
</table>
Which of the metabolites, if any, is responsible for the skin rash?

The skin rash is caused by a benzylic sulfate formed in the skin while most of the covalent binding in the liver is caused by a quinone methide. (Chem. Res. Toxicol. 21: 1862, 2008)
Covalent binding of nevirapine and the rash are inhibited by a topical sulfotransferase inhibitor. (Chem. Res. Toxicol. 26: 410, 2013)

Power of a valid animal model

- Allowed us to definitively determine what chemical species is responsible for the IDR.
- We found that the skin can form reactive metabolites mediated by sulfotransferase, and there are other drugs that cause serious skin rashes that have the potential to form reactive sulfate metabolites.
- Allowed us to test the basis for the p-i hypothesis: it is false
- Allowed us to test the involvement of various cells in the immune response.
- Suggested a mechanism by which reactive metabolites can induce an immune response; specifically, inflamasome activation.
Attempts to Develop an Animal Model of Idiosyncratic Drug-Induced Liver Injury (IDILI)

For many years we tried to develop an animal model of IDILI.

Drugs also produce reactive metabolites in animals that covalently bind to proteins, i.e. signal 1.

If the Danger Hypothesis is correct it should be possible to produce an animal model by activation of antigen presenting cells, i.e. signal 2.

We tested multiple drugs and tried multiple ways, e.g. stimulation through toll-like receptors with LPS, co-treatment with cytotoxic drugs, etc, but nothing worked.

This is consistent with the clinical observation that patients with inflammatory conditions such as ulcerative colitis are not at increased risk.

Most drugs that cause IDILI do not cause liver injury in rodents, but amodiaquine causes mild, delayed onset liver injury in mice. (J Immunotoxicol 12(3): 247, 2015)
Amodiaquine is bioactivated to an iminoquinone that can be trapped with glutathione.

Depletion of glutathione should increase covalent binding and liver injury, but it did not increase binding, and it paradoxically prevented liver injury.
It should be possible to increase liver injury by immunization with amodiaquine-modified hepatic proteins, but immunization prevented the injury!

J. Immunotoxicol. 12: 361, 2015

Immunization with amodiaquine-modified hepatic proteins markedly increased the number of myeloid-derived suppressor cells and T regulatory cells.
The dominant immune response in the liver is immune tolerance.  
(Heymann and Tacke, Nature Reviews 13: 88, 2016)

It might be possible to develop an animal model by blocking immune tolerance.

- Drugs called checkpoint inhibitors that block immune tolerance have been developed to treat cancer.
- PD-1 is expressed on activated T cells, B cells, and macrophages. It negatively regulates T cell receptor signalling.
- CTLA-4 is expressed on T cells and competes with CD28 thus blocking one of the major components of signal 2.
Treatment of PD-1/−/− mice with amodiaquine + anti-CTLA-4 lead to much greater injury that did not resolve with continued treatment. (Hepatology 61:1332, 2015)

It also led to histology characterized by piecemeal necrosis similar to IDILI in humans, and an increase in bilirubin.
Immune Response to Amodiaquine

- There is a compensatory increase in PD-1 and CTLA-4 in wild type animals as well as Tregs, and an even greater increase in Tregs in PD-1⁻/⁻ animals.
- There is also an increase in CD8 T cells that express granzyme B and perforin in PD-1⁻/⁻ animals.

Depletion of CD8 T cells prevents the liver injury.
Does this model unmask the ability of other drugs to cause IDILI? (Chem Res Toxicol 28:2287, 2015)

The answer is yes, although the injury is less with other drugs, and it resolves with continued treatment with the drug.

- nevirapine
- Isoniazid
- Carbamazepine
- Green Tea Extract
- Troglitazone
- Tolcapone

Does this model differentiate drugs that cause IDILI from those that do not?
Although the injury observed with entacapone could be viewed as a false +, there is a clear difference in the immune response; tolcapone resulted in a marked increase in Tregs and MDSCs, but entacapone did not. This tolerogenic response may be a better predictor of IDILI risk than injury.

How do reactive metabolites activate the immune system?

➢ The nevirapine model provided a possible clue. The model is similar to contact hypersensitivity in that a reactive molecule binds to proteins in the skin and induces an immune response. In the contact hypersensitivity model, mice that are deficient in components of the inflammasome are resistant.

➢ Inflammasomes, e.g. NLRP3, are present in macrophages, and when activated, produce the inflammatory cytokine IL-1β.

➢ We have found that several drugs that are chemically reactive or are oxidized to reactive metabolites by myeloperoxidase, which is present in macrophages, activate inflammasomes leading to the production of IL-1β.
Telaprevir is associated with toxic epidermal necrolysis while boceprevir is not. Dimethyl fumarate causes hypersensitivity, but ethacrynic acid does not. (Chem Res Toxicol 27: 949, 2014)

Fig 1. Telaprevir activates inflammasomes in THP-1 cells with production of IL-1β, which is inhibited by ZVAD, while boceprevir does not. Likewise dimethyl fumarate activates inflammasomes while ethacrynic acid does not.

Amodiaquine is oxidized to a reactive metabolite by macrophages and activates inflammasomes.
Clozapine causes agranulocytosis and olanzapine does not. Only clozapine activates inflammasomes.

But how would you test drugs that required P450 to form a reactive metabolite?

- If the reactive metabolite causes hepatocyte damage with the release of danger-associated molecular pattern molecules (DAMPs), then these DAMPs may be responsible for activation of macrophages in the liver.

- This was tested by incubation of hepatocytes with drugs that cause IDILI and then incubating macrophages with the supernatant from these incubations.
Incubation of macrophages with nevirapine did not activate inflammasomes, but incubation of macrophages with the hepatocyte supernatant did. (Chem Res Toxicol 30: 1327, 2017)

Summary

➢ There is substantive evidence that most IDRs are immune mediated, and most, but not all, are caused by reactive metabolites.

➢ The immune system is very complex with many redundant and compensatory mechanisms; therefore, it is difficult to predict the outcome of immune system activation.

➢ The dominant immune response to drugs that can cause IDRs appears to be immune tolerance, especially in the liver.

➢ Impairment of immune tolerance led to the first animal model of IDILI with characteristics similar to IDILI in humans. This makes it possible to test hypotheses that could not previously be tested.
Summary (continued)

- There is preliminary evidence that at least some IDRs involve the production of DAMPs that activate inflammasomes, which in turn initiates an immune response.
- With a much better understanding of the mechanisms of IDRs it is likely that better screening tests that predict IDR risk can be developed.
- However, given the complexities of the immune response it may never be possible to completely prevent IDRs.