New Methods to Predict DILI Risk

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What is the mechanism of most IDRs?

- Mechanistic studies of IDILI are difficult, and almost nothing is known with certainty.
- There is evidence that most idiosyncratic drug reactions, including IDILI, are immune-mediated (general characteristics, HLA associations, + lymphocyte transformation test, anti-drug antibodies, etc.)
- Other hypotheses:
  - Mitochondrial Injury
  - Bile Salt Exporter Protein Inhibition
  - ER Stress/Unfolded Protein Response
Using assays to predict risk before knowing whether the basic premise is correct is putting the horse before the cart.
How Can Mechanistic Hypotheses Be Tested?

• In theory, the best way would be to do controlled studies in humans, including trying to cause IDRs, but that is impossible.

• Animal models are essential for controlled experiments to test hypotheses. Animals do not predict IDRs, but neither do humans.

• Animals can have IDRs, but they are just as idiosyncratic in animals as in humans; the incidence has to be high for the model to be practical.

• To be of use the model must involve essentially the same mechanism as the human IDR; therefore, the characteristics should be the same; e.g. delayed onset and for liver injury, a hepatic infiltrate of mononuclear cells, not neutrophils.

• Findings in animal models should be validated by comparison with drug-induced changes in humans.
Attempts to Develop an Animal Model of Idiosyncratic Drug-Induced Liver Injury (IDILI)

• If IDILI is immune mediated it might be possible to develop animal models by stimulation of the immune system.

• For decades we tried to develop animal models of IDILI and other IDRs with several drugs (clozapine, amodiaquine, isoniazid, nevirapine, procainamide, dapsone, penicillamine, felbamate, propylthiouracil, ticrynafen, carbamazepine, minocycline, vesnarinone, etc.) by trying to stimulate antigen presenting cells with LPS, poly-IC, and other TLR agonists, co-treatment with cytotoxic drugs, etc. But these attempts have been failures. The immune system always seemed to be able to down regulate the response.

• This fits with the clinical observation that inflammatory conditions such as inflammatory bowel disease do not appear to be a major risk factor.
The dominant response to drugs that can cause IDILI appears to be immune tolerance

- Mild IDRs are always more common than serious IDRs and represent a “signal” that the drug may cause serious IDRs, but the ratio between mild and serious IDRs varies markedly from drug to drug.
- The basic mechanism of the mild reactions appears to be the same as serious IDRs, e.g. mild lumiracoxib-induced liver injury is associated with the same HLA as severe injury.
- If the injury is immune mediated and resolves despite continued treatment, this “adaptation” must involve immune tolerance.
If the dominant response to drugs that cause idiosyncratic liver injury is immune tolerance, it might be possible to develop an animal model by blocking immune tolerance.

- Drugs called checkpoint inhibitors that block immune tolerance are being developed to treat cancer.
- PD-1 is expressed on activated T cells, B cells, and macrophages. It negatively regulates TCR signals.
- CTLA-4 is expressed on T cells and competes with CD8 for binding to CD80 and CD86 on antigen presenting cells to inhibit immune responses.
Treatment of PD-1\(^{-/-}\) mice with amodiaquine + anti-CTLA-4 leads to liver injury and piecemeal necrosis that looks like IDILI in humans.
AQ treatment increases the number of PD-1$^+$ and CTLA-4$^+$ T cells and in PD-1$^{-/-}$ mice cotreated with anti-CTLA-4 increases cytotoxic T cells.
Depletion of CD8 T cells prevents amodiaquine-induced liver injury.
Checkpoint Block Also Unmasks INH-Induced Liver Injury
And Nevirapine-Induced Liver Injury
This model differentiates troglitazone, which causes IDILI, from pioglitazone, which does not.
Although immune tolerance is impaired, there is partial compensation by an increase in Tregs.
Implications of this Model

• It supports the hypothesis that the major response to drugs that can cause serious IDRs is immune tolerance, and it demonstrates how the immune system tries to compensate for the loss of PD-1 and CTLA-4.

• This model will allow us to test hypotheses that we have never been able to rigorously test before.

• It should allow the prediction of which drug candidates will cause idiosyncratic liver injury, but it probably will not be perfect.

• It predicts that checkpoint inhibitors will increase the risk of IDRs caused by other drugs, and this appears to be the case, e.g. vemurafenib.
Testing the hypothesis that mitochondrial injury is a common mechanism of IDILI

- Mitochondria play a major role in controlling cell death, and mitochondrial injury is an attractive hypothesis for the mechanism of IDILI, either directly or by stimulating an immune response.
- Mechanisms of mitochondrial injury include inhibition of fatty acid metabolism, inhibition of mitochondrial DNA synthesis, inhibition of mitochondrial protein synthesis, inhibition of the mitochondrial electron transport chain, and uncoupling oxidative phosphorylation.
- There are drugs and other agents that can affect mitochondria through all of these mechanisms, yet with the exception of valproate, they rarely cause typical IDILI, and even valproate-induced IDILI is rather unique.
A common test to predict DILI risk is inhibition of the mitochondrial electron transport chain

- Metformin inhibits complex 1 in the electron transport chain and causes lactic acidosis, but it does not cause IDILI, and it does not even appear to increase the risk of IDILI with co-administered drugs.

- Rotenone is the classic inhibitor of complex 1. In comparison to metformin it binds more tightly and appears to increase ROS formation so its effects could be different from those of metformin.
Testing the Mitochondrial Injury Hypothesis

- Rotenone inhibits complex 1, and isoniazid or its metabolites inhibit complex 2.
- In vitro the combination is synergistic and cytotoxic to hepatocytes.
- We tested the combination in vivo. In vivo the combination is also synergistic and lethal in wildtype mice, but it does not cause liver injury as predicted by the in vitro experiment.
- In the impaired immune tolerance model, lower doses of rotenone did not significantly increase isoniazid-induced liver injury.
- Although inhibition of the mitochondrial electron transport chain can cause lactic acidosis, it does not appear to be a common mechanism of IDILI.
Inflammasome Activation

• Contact hypersensitivity is an immune response to xenobiotic covalent binding in the skin.

• Animals with impaired inflammasome activity are resistant to contact hypersensitivity.

• We have found that several reactive agents that cause IDRs activate inflammasomes.
Can Inflammasome Activation Differentiate Reactive Species That Are Likely to Cause IDRs?

- Telaprevir
- Dimethyl fumarate
- Boceprevir
- Ethacrynic acid
Telaprevir is associated with TEN while boceprevir is not; dimethyl fumarate causes hypersensitivity reactions and ethacrynic acid does not.

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.
Clozapine vs Olanzapine

- Clozapine activates inflammasomes but olanzapine, which has a very similar structure and forms a similar reactive metabolite does not.
Inflammasome Activation by Amodiaquine

Amodiaquine Treated THP-1 Macrophages

IL-1B Sec (pg/ml)

Sample (ug/ml)

Med  DMSO  AQ-2  AQ-5  AQ-10  AQ-10z
Testing drugs that are bioactivated by P450

- Most drugs that cause IDILI are not bioactivated by THP-1 cells or other macrophages; how could they be tested?
- It is more likely that drugs are bioactivated by hepatocytes that, in turn, release DAMPs, which activate antigen presenting cells and other macrophages.
- This was tested by incubation of nevirapine with hepatocyte spheroids for a week and then THP-1 cells were incubated with the supernatant from these hepatocytes.
Hepatocytes release DAMPs that can activate inflammasomes

A  Nevirapine only

B  Hepatocyte supernatant

![Graph A: IL-1β levels for control and different concentrations of Nevirapine.](image)

![Graph B: IL-1β levels for control and various treatments.](image)

- Control
- NVP 10 μM
- NVP 30 μM
- NVP 100 μM
- NVP 100 μM + ZVAD
- NVP 100 μM + 1-phenylethanol
- NVP 100 μM + 1-amino-2-naphthol
Inflammasome activation differentiates troglitazone from pioglitazone

Drug only  

Hepatocyte supernatant
And partially differentiates tolcapone and entacapone
Conclusions

- Although little is known for certain, there is compelling evidence that most IDRs are immune mediated.
- It is important that hypotheses be tested before they are widely used to predict risk.
- We have developed an animal model involving impaired immune tolerance that can be used to test hypotheses.
- It does not appear that inhibition of the mitochondrial electron transport chain is an important mechanism of IDILI.
- This animal model may be useful for predicting risk, but it is unlikely to be perfect.
- The release of DAMPs that activate inflammasomes may be an important mechanism, and this may be the basis for predicting IDILI risk, but it must be further tested including testing whether this also occurs in humans.
- The study of the basic immune response to drugs, first in animals and then in humans, is critical for developing new predictive biomarkers.