Inflammation as a Source of Variability in Drug Disposition and Response

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The Clinical Story

PATIENTS often exhibit large differences in drug disposition and response

Patients frequently have a number of other underlying conditions/diseases.

Inflammatory responses occur commonly in patients with a variety of acute and chronic diseases.

Clinical reports of dramatic increases in toxicity and blood levels of theophylline during infection.
8-10 fold increase in plasma concentrations of propranolol in Rheumatoid arthritis and Crohn’s patients with “active disease”.

ESR = erythrocyte sedimentation rate


Similar Inflammatory Responses in Experimental Models

Acute “inflammatory” response induced by low dose endotoxin in healthy volunteers decreases drug metabolism.

Decreased oral clearances of antipyrine, hexobarbital, and theophylline in 6 healthy males after administration of endotoxin (20 U IV 24 hr prior to drug) as compared to saline.

Shedlofsky et al. J Clin Inv. 1994
Endotoxin and pro-inflammatory cytokines decrease expression of cytochrome P450s in primary cultures of human hepatocytes.

FIG. 1. Effects of cytokines on P450 mRNA expression in human hepatocytes. Cells were treated with saline (Control), LPS (10 g/ml), IL-6 (10 ng/ml), TNF (10 ng/ml), IFN (10 ng/ml), TGF (10 ng/ml), or IL-1 (5 ng/ml) for 24 h

Aitken & Morgan DMD 2007
- Downregulation of numerous drug metabolizing enzymes in liver
- Altered plasma protein binding
  - $\uparrow$ alpha$_1$ acid glycoprotein (AAG)
- Other mechanisms?
Many drugs actively transported
Absorption/ Distribution/ Excretion

Changes in expression & activity of transporters affects the PK & PD of these drugs.
Regulation of Transporters in disease not as well understood.
ABC Efflux and SLC Uptake Transporters Expressed in Epithelial Membranes of Intestine, Brain, Placenta, Liver & Kidney.

Disease-induced changes will impact absorption, distribution into tissues and elimination of drug substrates.
Effect of Inflammatory Disease on Drug Transporters?

- Impact on expression?
- Tissues affected?
- Impact on drug disposition?

**Infection / Inflammatory Response:**
- Induced in rodents (rat, mouse) with bacterial endotoxin (LPS); viral mimetic (Poly I:C); or live malaria parasite (P. beghei).
Effect of Endotoxin-induced Inflammation on Transporters in Liver

Many ABC and SLC drug transporters are downregulated in rodents after administration of the bacterial endotoxin - LPS

mRNA expression in mice 6 hr after 5 mg/kg LPS; % saline control; n=6

Impact on Drug Disposition

↓ Biliary Clearance of Doxorubicin in Endotoxin (LPS)-treated mice.

↓ mdr1/ PGP expression.

In vivo administration of IL-6 to mice

Downregulation of PGP, MRP2, BSEP, OATP1a1, OATP1a4 in liver consistent with downregulation seen after LPS administration.

Int Immunopharmacol. 2001; 1: 189-99

Pro-inflammatory cytokine IL-6 downregulates several drug transporters in primary cultures of human hepatocytes
Altered Expression of several ABC- & SLC-Transporters in intestine of Endotoxin-treated Rats
Expression associated with reduced Basal to Apical Efflux of Mdr1 (Pgp) and Mrp2 Substrates in intestinal segments.

Decreased B→A efflux increased (1.5 X) Net Absorption of PGP substrate, Amiodarone in LPS group.

Inflammatory episode – diarrhea increase plasma concentrations of Tacrolimus in patients reportedly due to decreased activity of PGP in intestine.

Lemahieu et al., Am J Transpl. 2005

Relative activities of CYP3A4 and PGP based on urinary & breath recovery of 13C after 13C-aminopyrin (po) and 14C recovery after 14C- erythromycin (po & iv).
KIDNEY

Altered Expression of ABC- and SLC-Transporters in KIDNEY of Endotoxin-treated Rats
Oat1/ Oat3 downregulation associated with decreased creatinine clearance and PAH renal secretion in endotoxin-treated rats.
Effect of 24 hr LPS treatment (given via i.c.v.) on brain $^{3}$H-digoxin accumulation in mice and rats

Mice | Rats
---|---
0 | Saline
50 | LPS
100 | $^*$
150 | $^*$
200 | $^*$
250 |

DPM g$^{-1}$ tissue as % of saline control

↑ $^3$H-Digoxin in brain of LPS-treated rodents

↓ mdr1a and oatp1a4 in brain of LPS-treated mice

Altered Expression of Pgp and Oatp1a4 in BRAIN of Endotoxin-treated Rodents

Increased CNS accumulation of morphine 3 glucuronide after brain injury in patients

K Renton  DMD 2008
& Critical Care Med. 2009

M3G: Substrate of MRP1 and Oatps

Distribution of morphine and glucuronides After brain injury caused by hemorrhage.

Increased accumulation in CSF significantly Correlated to IL-6
Decreased expression of ABC-efflux and SLC-influx transporters in placenta of endotoxin-treated rats.

MRNA expression 12-18 hr post LPS (1 mg/kg) on GD17
Fetal Accumulation of Substrates in Endotoxin-treated rats

**Fetal Accumulation of PGP (ABCB1) Substrate-**

**Tc-99m-sestamibi**


**Fetal Accumulation of BCRP (ABCG2) Substrate-**

Glyburide

DMD. 2008;36:1944-50

Accumulation of 99 Tc-sestamibi in Fetus

Tissue/plasma glyburide concentration ratios (ng/g tissue) / (ng/mL plasma)

**Fetal Accumulation of Substrates in Endotoxin-treated rats**
Human Placenta

Effect of bacterial infection (chorioamnionitis- CAM) on protein expression of transporters in placenta.

Pre-term pregnancies (29 ± 2.5 weeks)

Controls: healthy term-matched pregnancies.
Results from western blot immuno-detection (mean ±SEM, n=14/group).

↓ Expression of important drug / steroid transporters
OTHER MODELS OF INFECTION & INFLAMMATION
**Viral Infections**

- Associated with induction of acute inflammatory response.
- Classic model is via admin. of the synthetic viral-like double stranded RNA PolyI:C

  - poly-IC activates Toll-like receptor 3 (TLR3) – which induces interferons whereas LPS activates Toll-like receptor 4 (TLR4); resulting in distinct pattern of cytokine release.
Impact of Poly I:C on Hepatic Expression of Transporters in Pregnant Rats.

mRNA expression 24 hr post Poly I:C
Decreased Expression of many ABC and SLC Transporters in Placentas of Poly I:C-treated Rats

**mRNA expression 24 hr post Poly I:C**
Impact on Maternal and Fetal levels of the anti-HIV protease inhibitor- Lopinavir (LPV)

LPV is a PGP and CYP3A substrate & highly protein bound. Several underlying mechanisms involved.
mRNA Expression of Transporters in Placenta from HIV (+) women managed with HAART.

Placenta obtained from HIV + women on PI- containing HAART (n=32) or uninfected controls (n=24). Preliminary results.

Disease or Therapy-induced changes?

** Many Protease Inhibitors activate PXR.
Parasitic Infection

Malaria:

- 247 million infections in 2008

Malaria in Pregnancy:

- Major global health problem with > 50 million pregnancies exposed yearly
  - Dramatic localized inflammatory response in placenta.
    - IUGR, low birth weight, prematurity, abortion, maternal and fetal deaths
  - Malaria activate TLR 2, 4 and 9: resulting in a unique activation of cytokine and cell signalling pathways.

Animal model: (Dr. Kevin Kain – University of Toronto)

- *P. Berghei* infection in pregnant Balb/c mice.
  - Infect mice with $10^6$ *P. Berghei* infected RBC on GD13
  - Collect maternal and fetal tissues on GD19

DMD 42:603-10; 2014
Impact of Malaria on Expression of Transporters in Maternal Liver

**Graph:**
- **Y-axis:** Fold-Change in Plasma Bile Acids (relative to control)
- **X-axis:** Genes (Cyp3a11, Abcc2, Abcg2, Abcc11, Abcb1a, Abcb1b, Abcc1, Abcc3)
- **Legend:**
  - Control
  - PbA Infected

**Annotations:**
- **Bcrp** and **Bsep** are highlighted
- **Mrp2** and **Mdr1b** are highlighted
- **Mrp3** is highlighted

**Statistical Significance:**
- ******* indicates statistical significance.
Impact of Malaria (P. Berghei) Infection on Expression of Transporters in Fetal Liver

Downregulation of Bcrp, Bsep, Cyp3A & Upregulation of mdr1b in fetal liver similar to that seen in Maternal liver.

![Graph showing expression levels of transporters in fetal liver](image)
MECHANISMS?
Involvement of Nuclear Factor Kappa B (NF-κB)

- Inducible transcription factor that plays a critical role in inflammation
  - Regulates over 200 genes involved in a variety of cellular processes.
- Activated by bacterial and virus antigens, pro-inflammatory cytokines and oxidative stress.
- Constitutively active in many chronic diseases associated with inflammation
In vivo administration of selective NF-κB inhibitor: PHA-408 (inhibits IκB kinase), suppressed cytokine induction and PXR downregulation in endotoxin (LPS)-treated mice
Inhibition of NF-κB suppresses downregulation of Cyp3A & ABC- efflux and SLC-uptake transporters in endotoxin-treated mice

Pre-treated with PHA-408
What if the Inflammatory response is controlled or decreased?

Intervention

Schmith & Foss, CPT, 2010
Clinical Disease-drug-drug interaction Tocilizumab (IL-6R antibody) and Simvastatin (CYP3A substrate) in patients with rheumatoid arthritis.

Exposure to Simvastatin and CRP levels significantly reduced after IL-6R antibody (10 mg/kg Day 8) in RA patients (n=12) Attributed to IL-6 mediated changes in CYP3A activity

Schmidtt et al. CPT 2011
Disease- DDI with **Sirukumab (IL-6 mAb)** in patients with active Rheumatoid Arthritis


Midazolam exposure ↓ 30-35%. Omeprazole exposure ↓ 37-47%. Warfarin exposure ↓ 18-20%

**Inflammation-mediated suppression of CYP3A, 2C19 & 2C9** reversed by anti-IL6 mAb
Conclusion

- Inflammation mediated alterations in the expression and activity of many important drug transporters and metabolizing enzymes.
- Changes associated with altered disposition of substrates
  - Many potential clinical consequences (Altered Absorption/Distribution/Clearance/Efficacy/Toxicity)
- NF-κB plays crucial role in regulation
- Info may be useful in prediction of disease-drug interactions.
- Biologics may add additional complexity
  * Induction or Resolution of inflammation may cause disease:ddI interactions
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Plasma Levels of Propranolol in Ketoprofen-Treated and Untreated Arthritic Rats

Piquette-Miller & Jamali; DMD 1995
What about Drug Targets?
Pharmacodynamics of Verapamil altered in Rheumatoid Arthritic Patients

Significant decrease in dromotropic effect in RA despite higher conc. Attributed to downregulation of β-receptors.