

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.

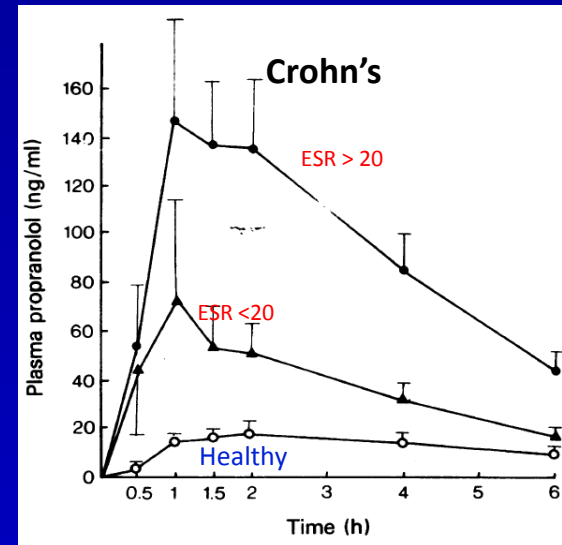
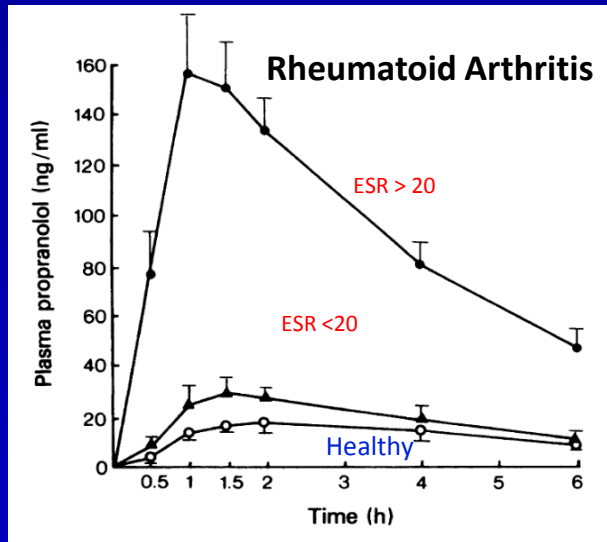


Inflammatory responses occurs commonly in patients with a variety of acute and chronic diseases

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

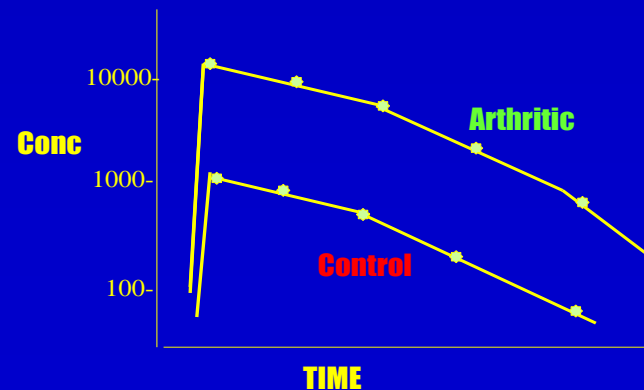


Schneider & Bishop. Br J Clin Pharm, 1979

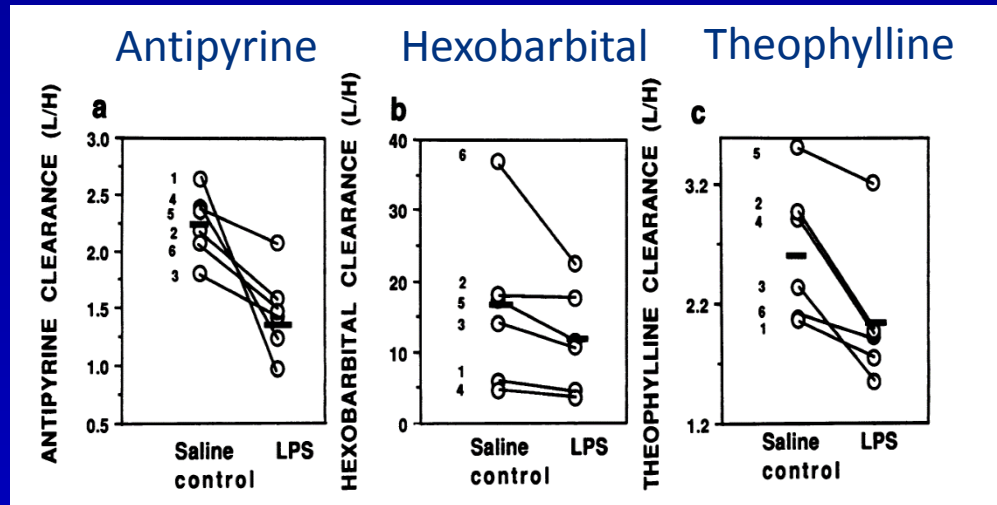
Similar Inflammatory Responses in Experimental Models

Piquette-Miller & Jamali. Pharm Res., 1993

Propranolol Arthritic vs Control Rats

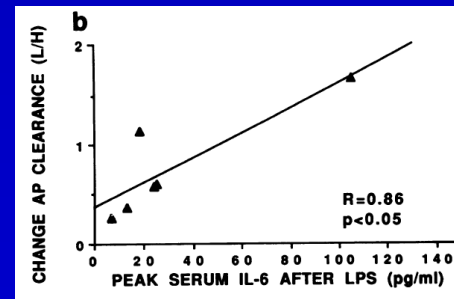


Acute “inflammatory” response induced by low dose endotoxin in healthy volunteers decreases drug metabolism.



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.

	Saline	LPS
IL-6 (pg/ml)	3 ± 1	52 ± 58
CRP (mg/dl)	0 ± 0	3.3 ± 0.5



Correlates to pro-inflammatory cytokine levels (IL-6 & TNF)

Endotoxin and pro-inflammatory cytokines decrease expression of cytochrome P450s in primary cultures of human hepatocytes.

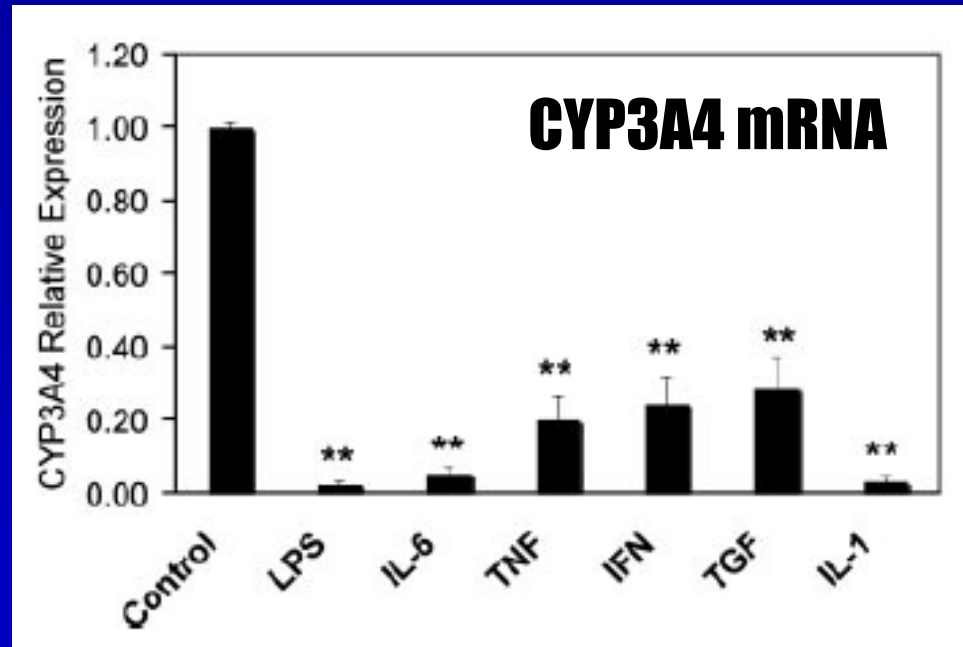


FIG. 1. Effects of cytokines on P450 mRNA expression in human hepatocytes. Cells were treated with saline (Control), LPS (10 g/ml), IL-6 (10 ng/ml), TNF (10 ng/ml), IFN (10 ng/ml), TGF (10 ng/ml), or IL-1 (5 ng/ml) for 24 h

- Downregulation of numerous drug metabolizing enzymes in liver
- Altered plasma protein binding
 - ↑ alpha₁ acid glycoprotein (AAG)
- Other mechanisms?



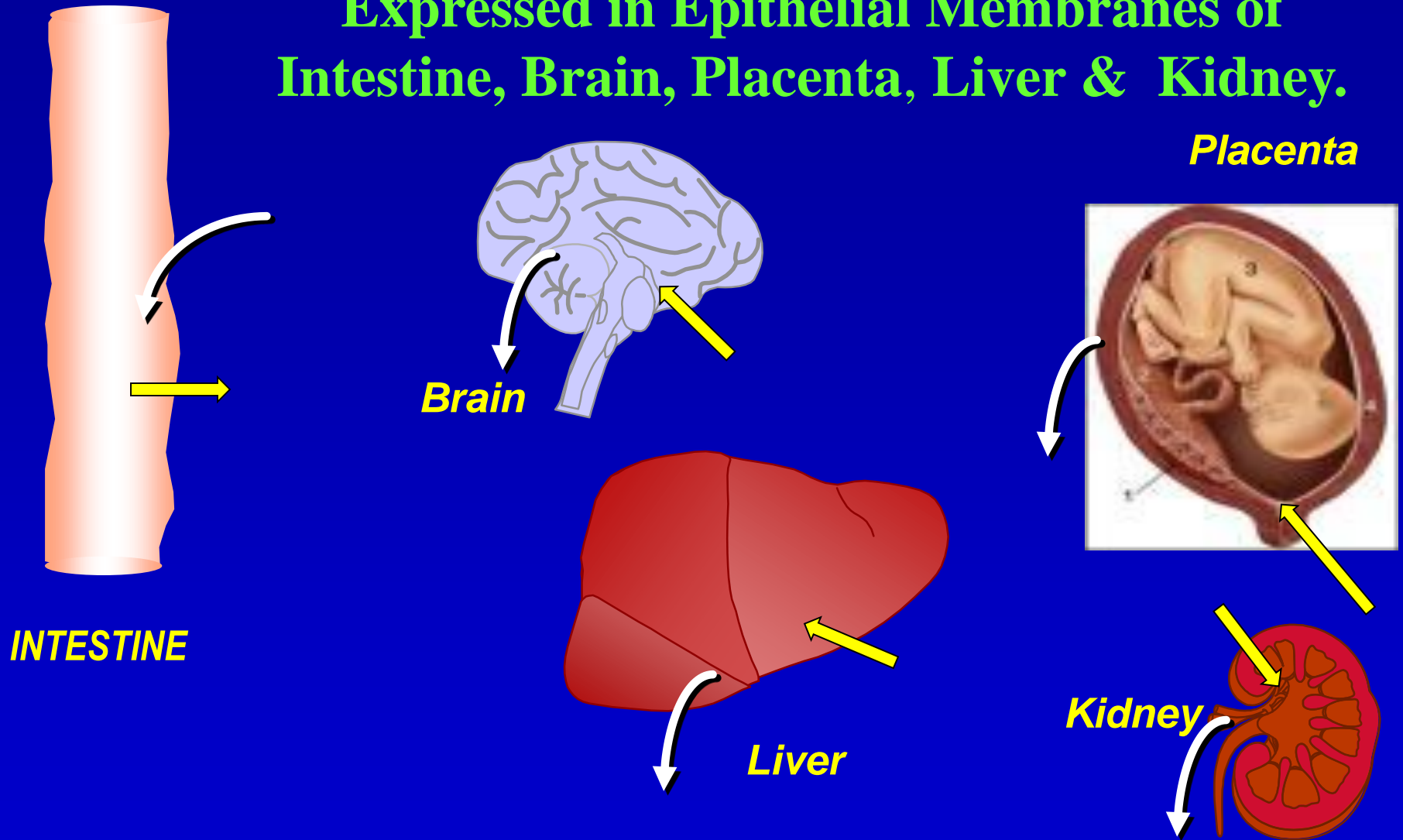
WHAT ABOUT DRUG TRANSPORTERS ?

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?

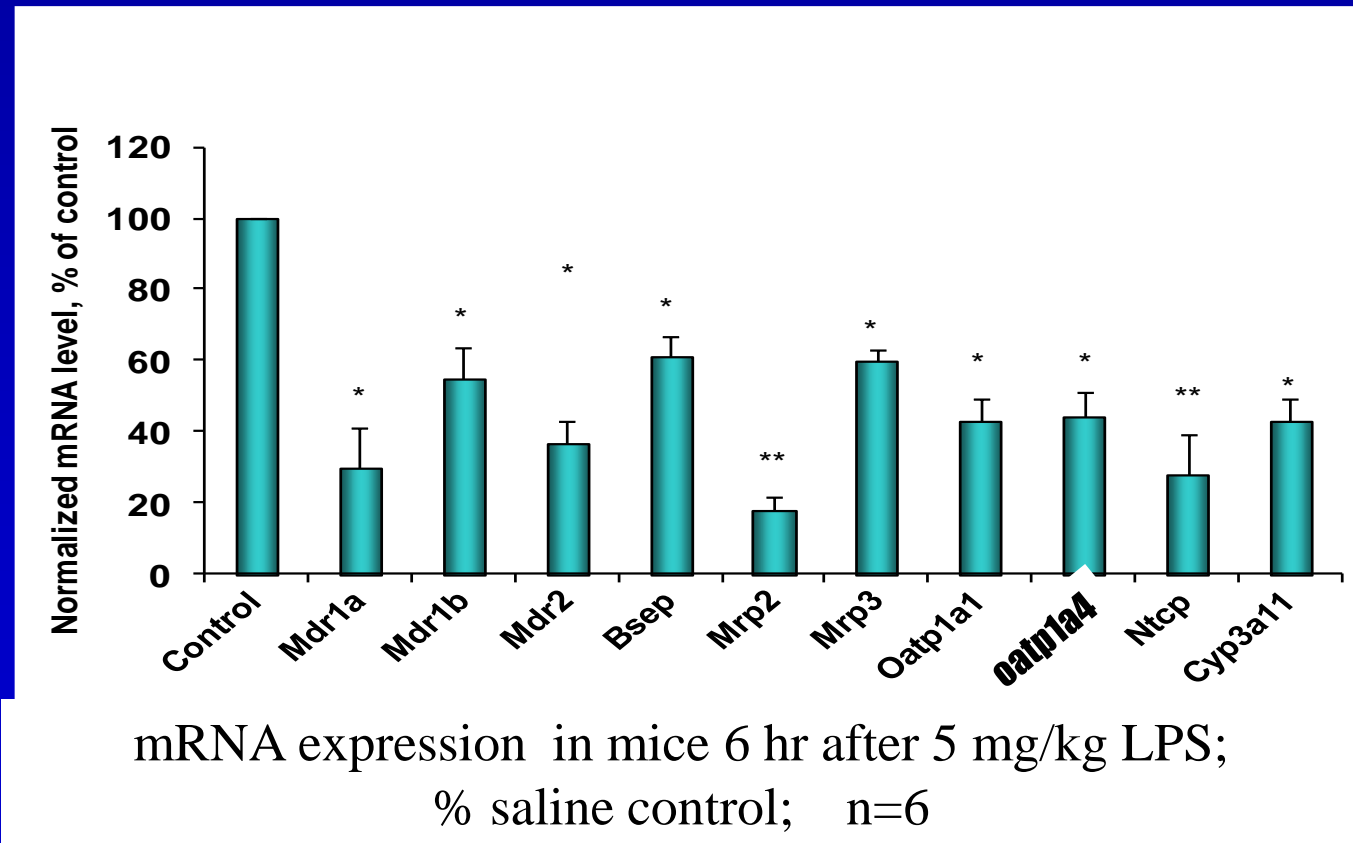


Infection / Inflammatory Response:

- Induced in rodents (rat, mouse) with bacterial endotoxin (LPS); viral mimetic (Poly I:C); or live malaria parasite (P. berghei).

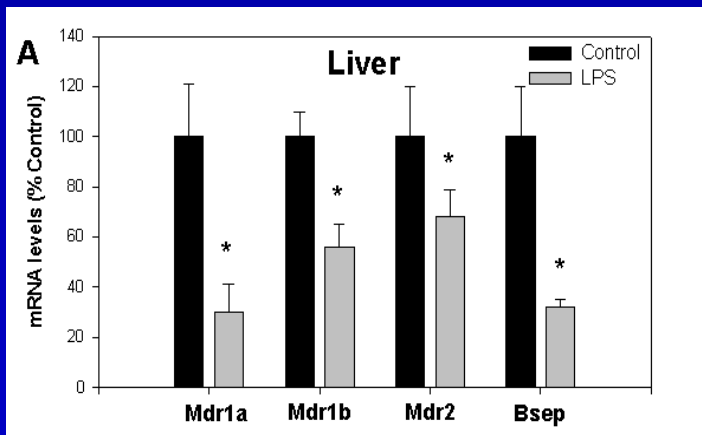
Effect of Endotoxin-induced Inflammation on Transporters in Liver

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

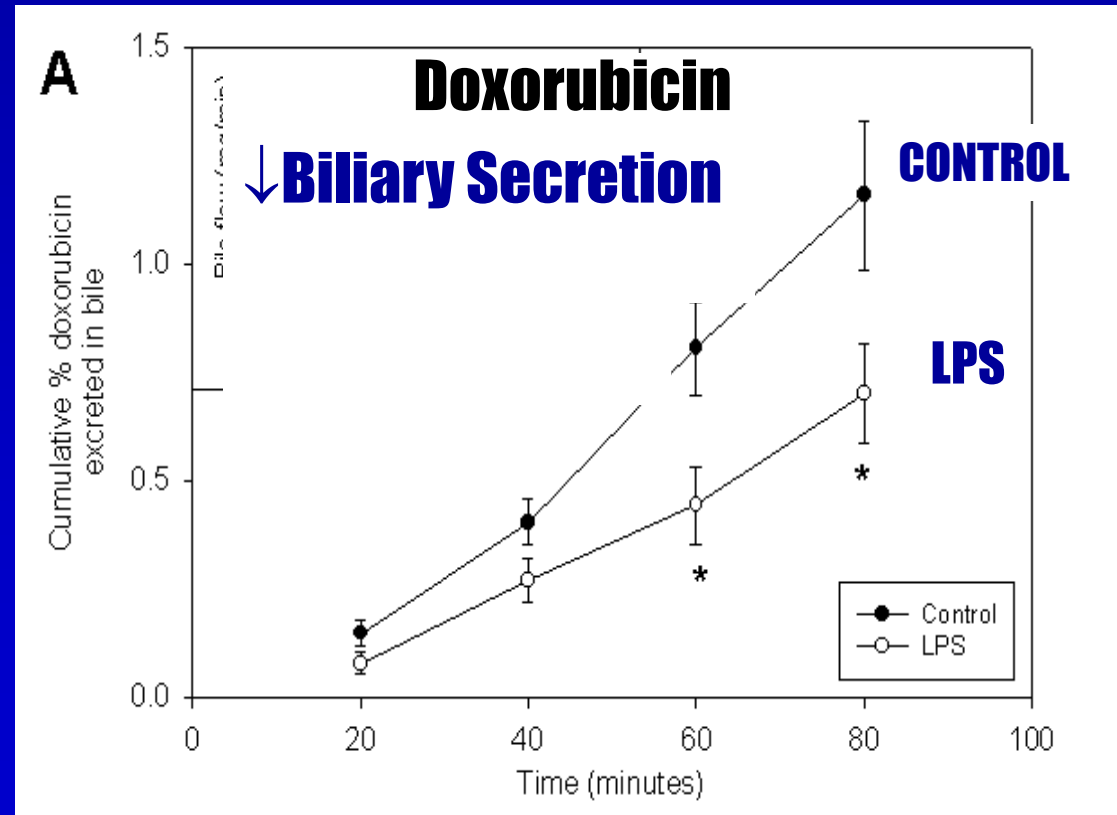


Impact on Drug Disposition

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

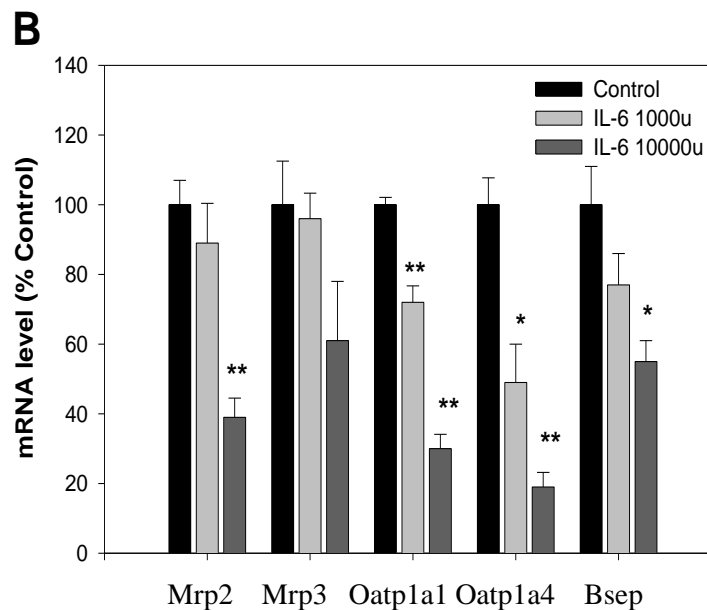
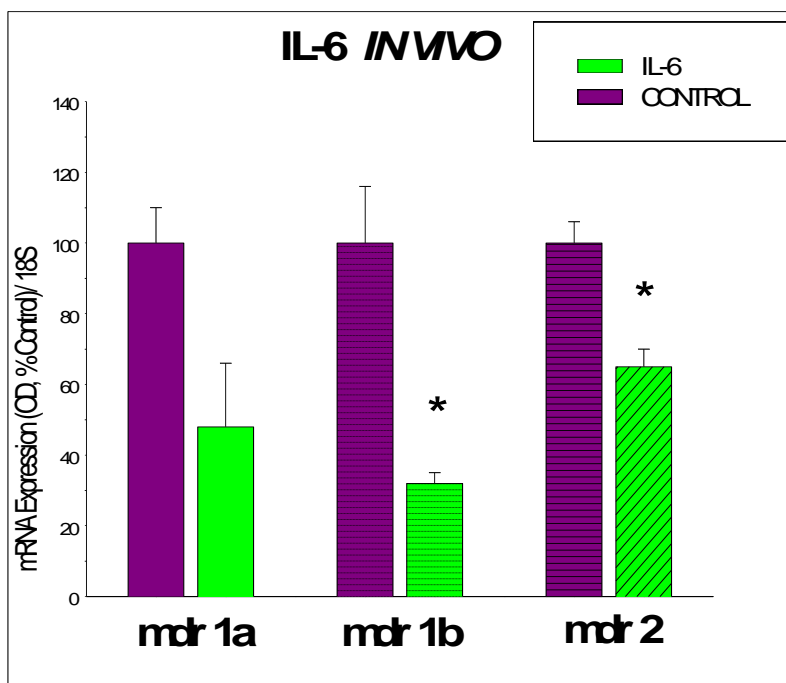


↓ **mdr1/ PGP expression.**

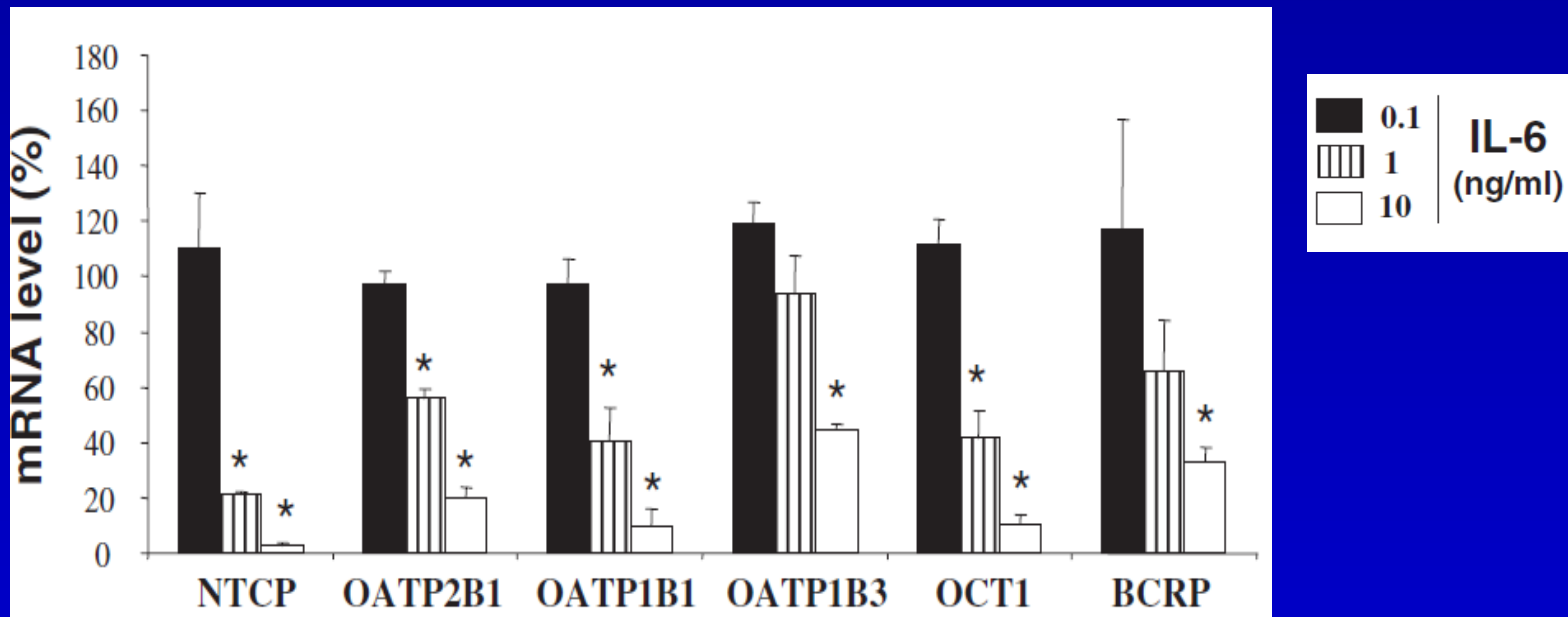


In vivo administration of IL-6 to mice

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



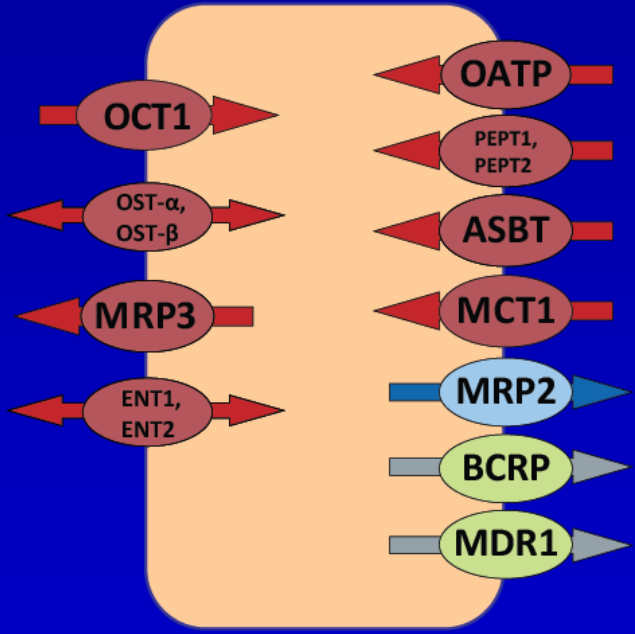
Pro-inflammatory cytokine IL-6 downregulates several drug transporters in primary cultures of human hepatocytes



INTESTINE

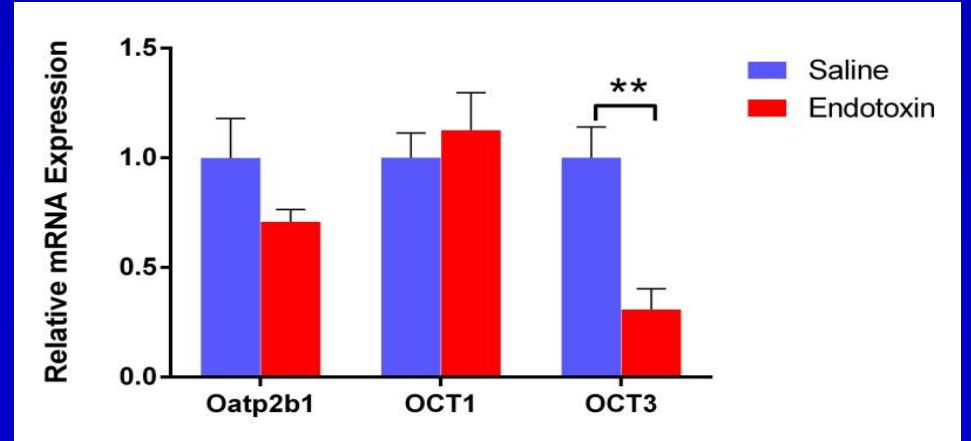
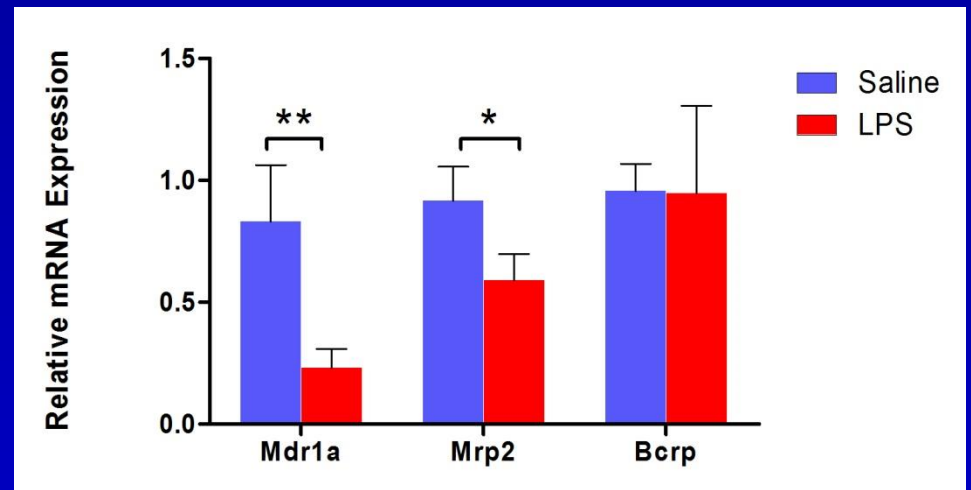
Small intestine

Blood Gut Lumen



- Transporters recommended in the 2012 FDA and EMA DDI guidance documents
- Transporters proposed for prospective or retrospective investigation
- Other transporters proposed that are not involved in DDI but may influence the PK or efficacy of a drug

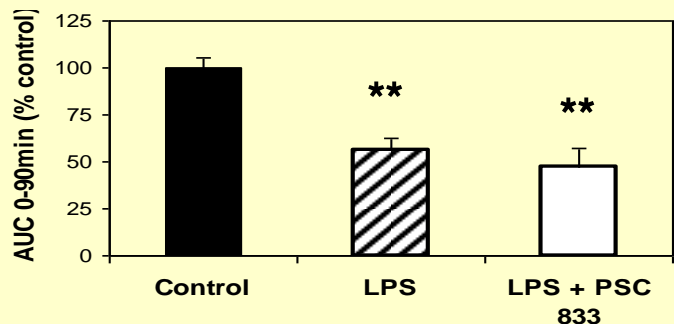
Altered Expression of several ABC- & SLC-Transporters in intestine of Endotoxin-treated Rats



↓ Expression associated with reduced Basal to Apical Efflux of Mdr1 (Pgp) and Mrp2 Substrates in intestinal segments

PGP

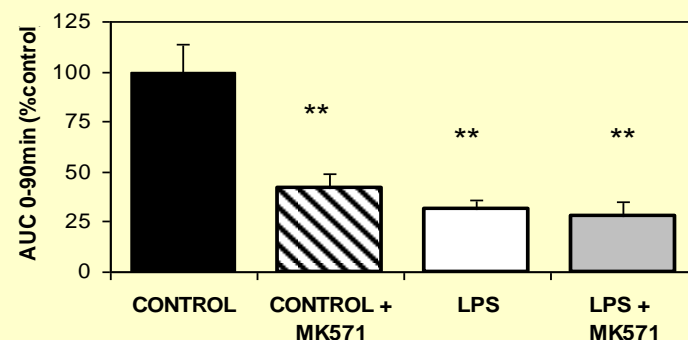
Digoxin



(B→A) Transport (Efflux)

MRP2

5-CF

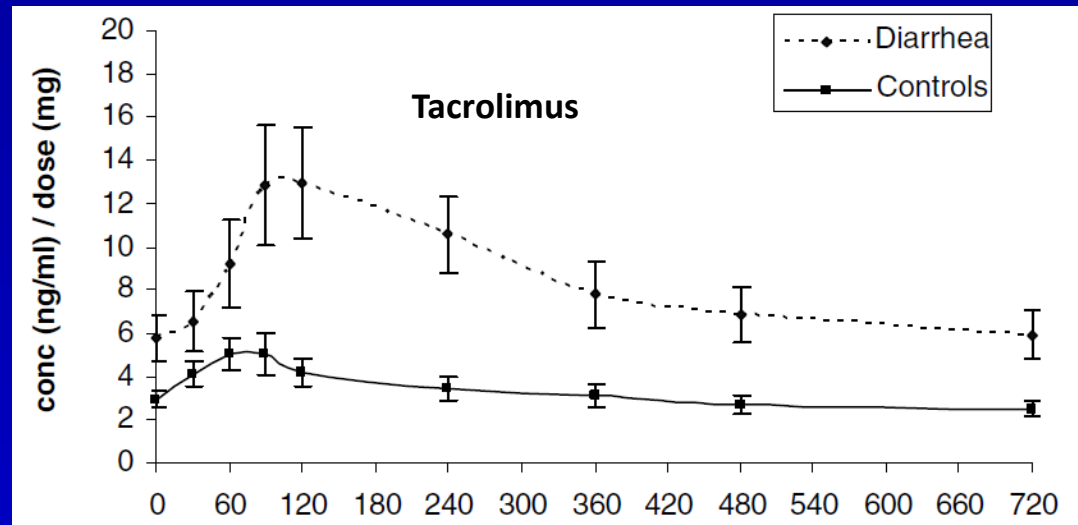
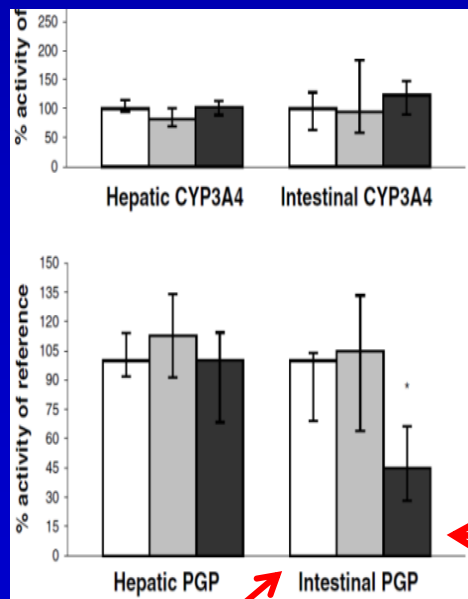


(B→A) Transport

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

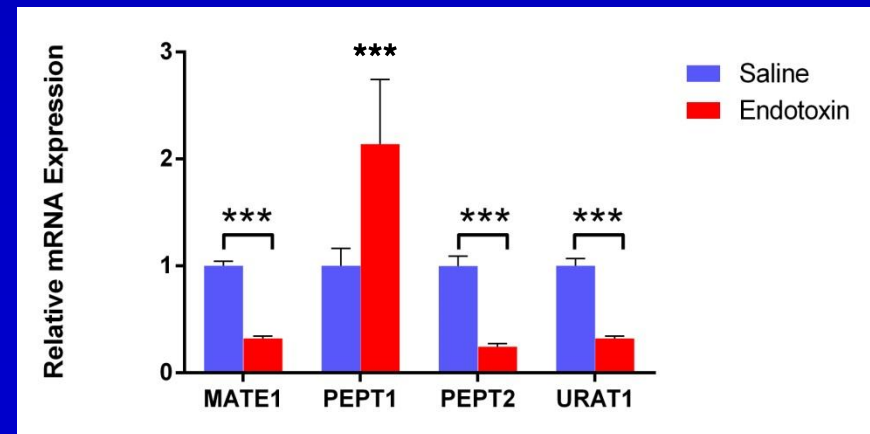
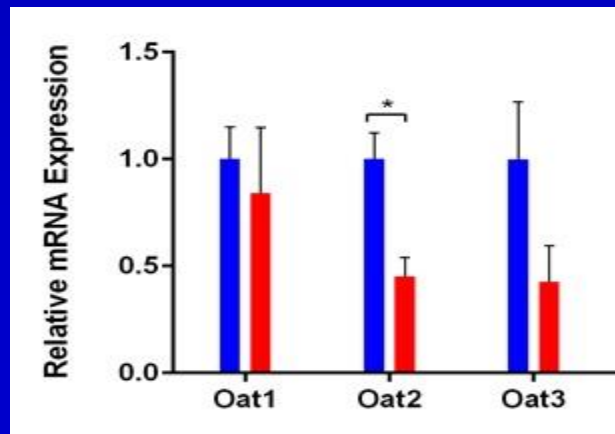
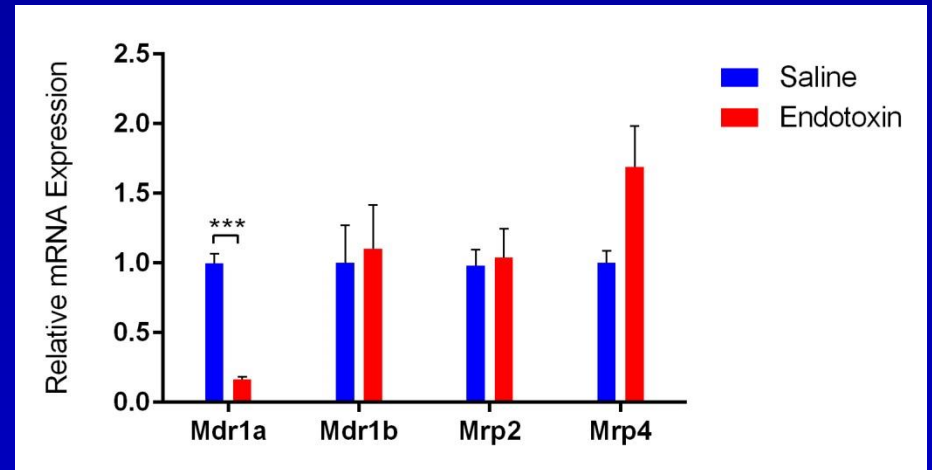
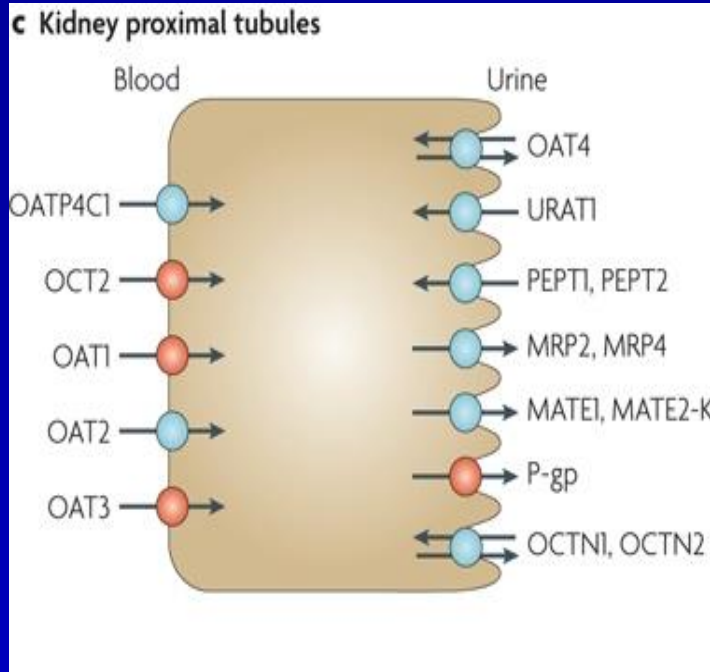


Relative activities of CYP3A4 and PGP based on urinary & breath recovery of ¹³C after ¹³C-aminopyrin (po) and ¹⁴C recovery after ¹⁴C- erythromycin (po & iv).

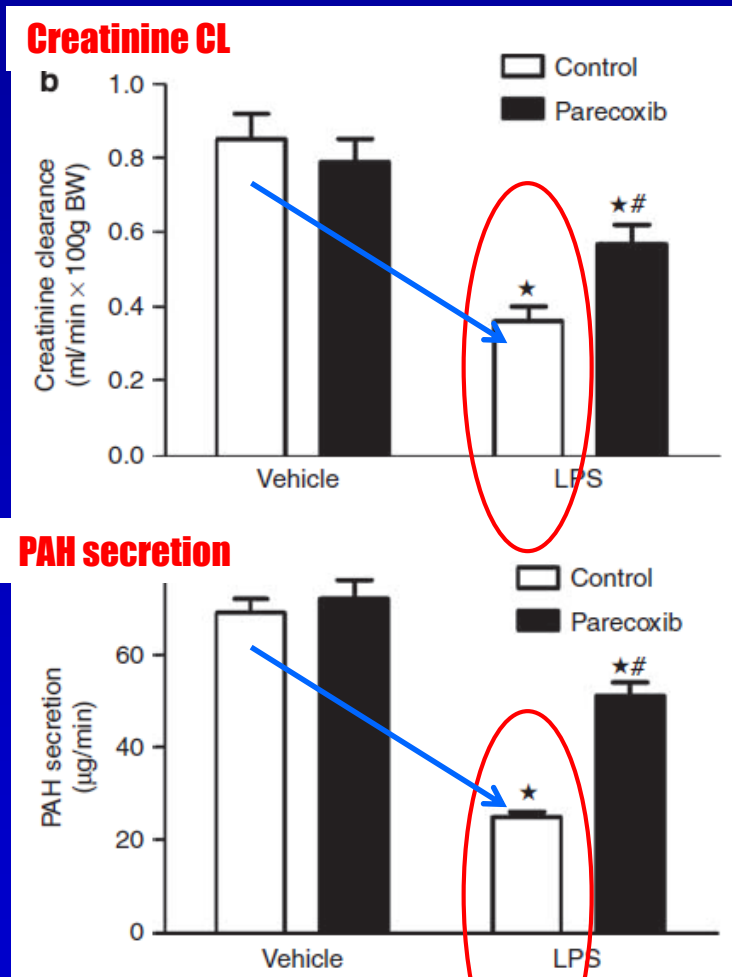
Healthy and Patient Controls

KIDNEY

Altered Expression of ABC- and SLC-Transporters in KIDNEY of Endotoxin-treated Rats



Hoher, Schmidt & Bucher, Kidney Int. 2009

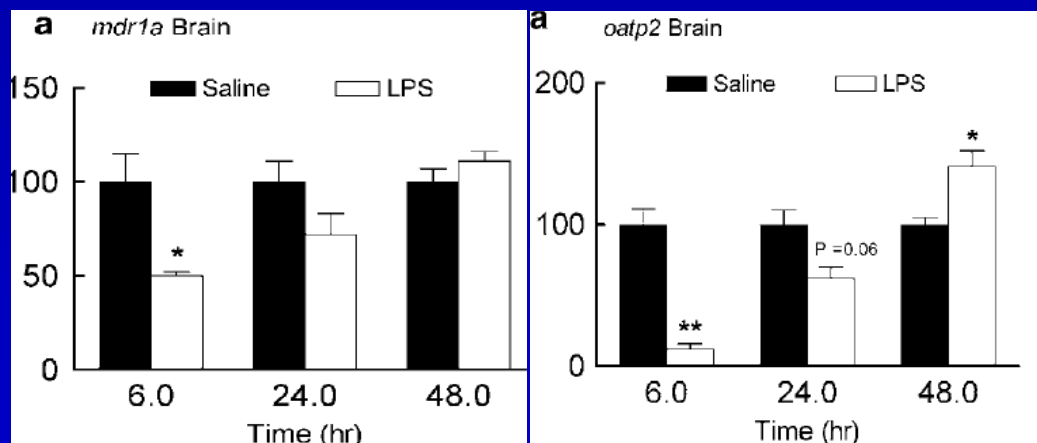


Oat1/ Oat3
downregulation
associated with
decreased creatinine
clearance and PAH
renal secretion in
endotoxin- treated
rats.

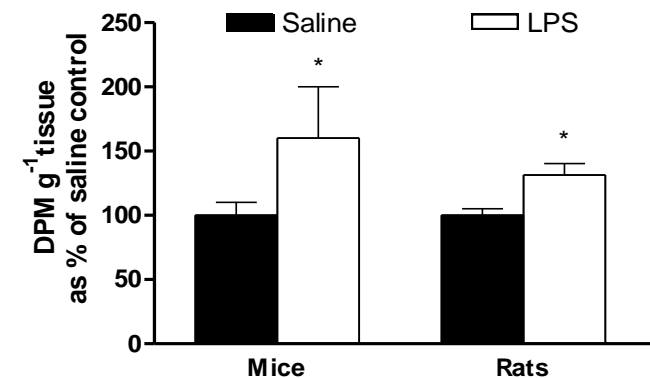
BLOOD-BRAIN BARRIER

↓ *mdr1a* and *oatp1a4* in brain of LPS-treated mice

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



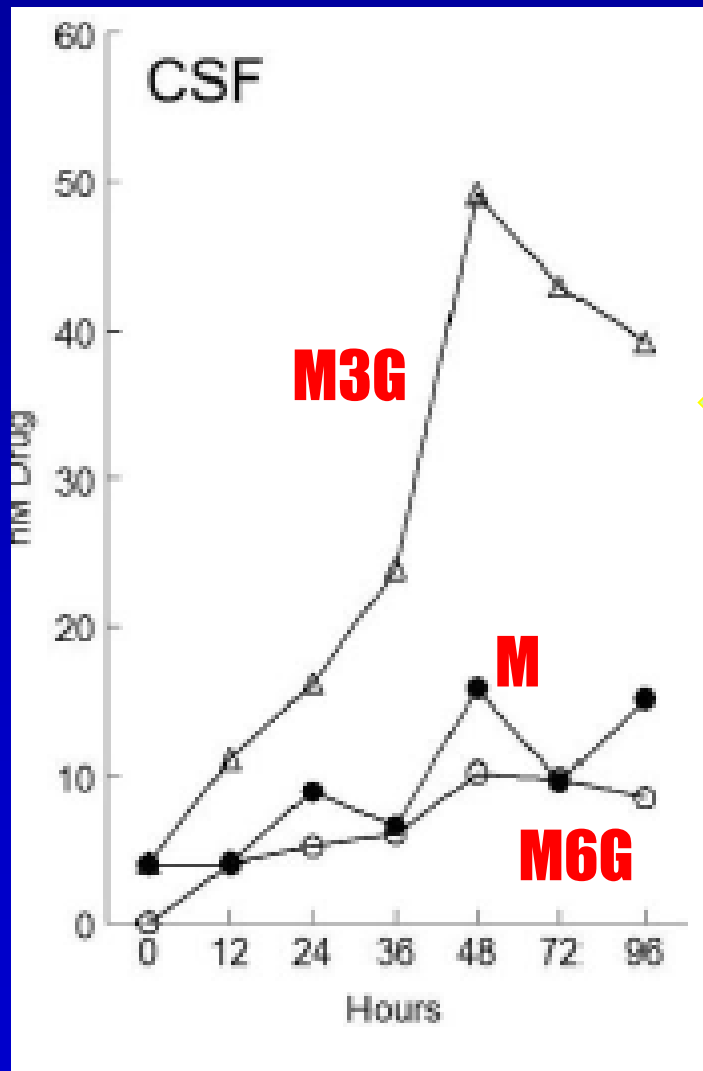
↑ ³H- Digoxin in brain of LPS-treated rodents



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

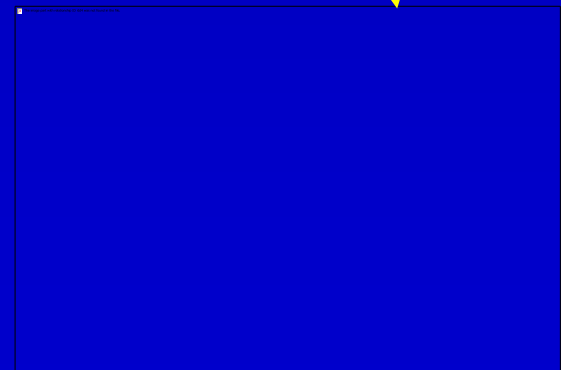
K Renton DMD 2008
& Critical Care Med. 2009

M3G: Substrate of MRP1 and Oatps



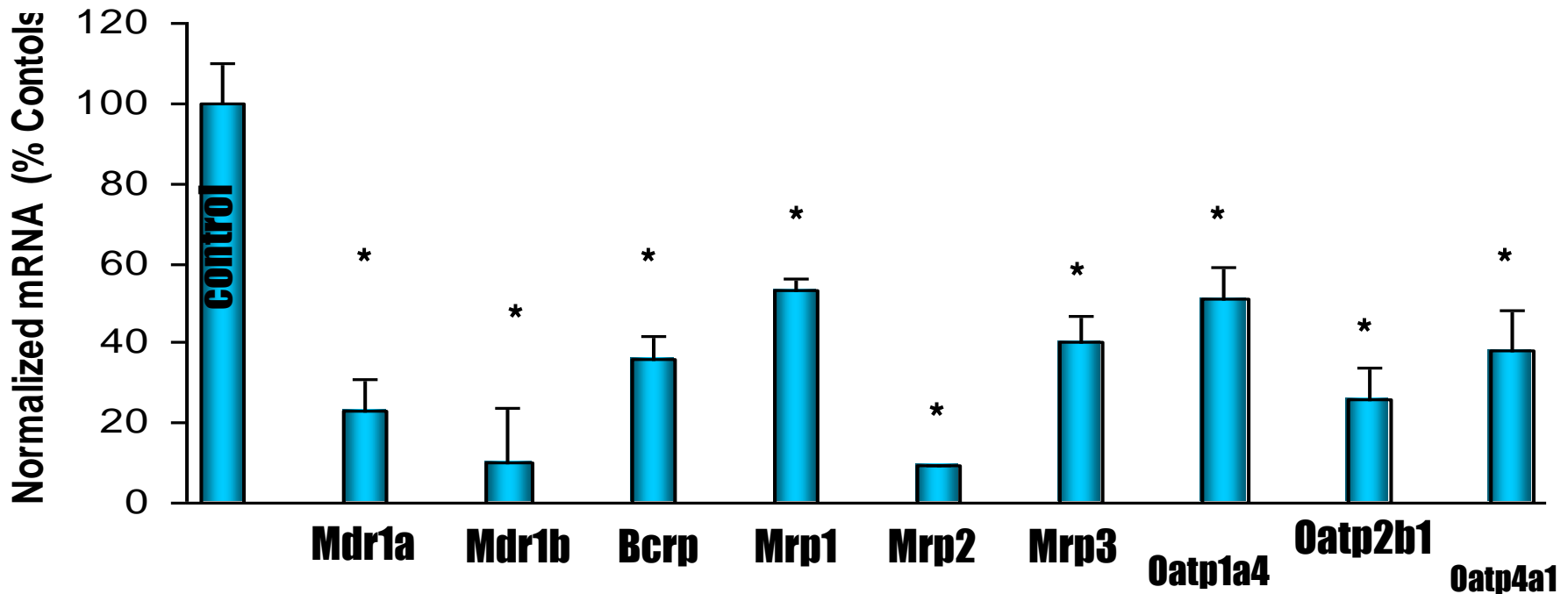
← Distribution of morphine and glucuronides After brain injury caused by hemorrhage.

Increased accumulation in CSF significantly Correlated to IL-6



PLACENTA

Decreased expression of ABC-efflux and SLC-influx Transporters in Placenta of Endotoxin-treated Rats

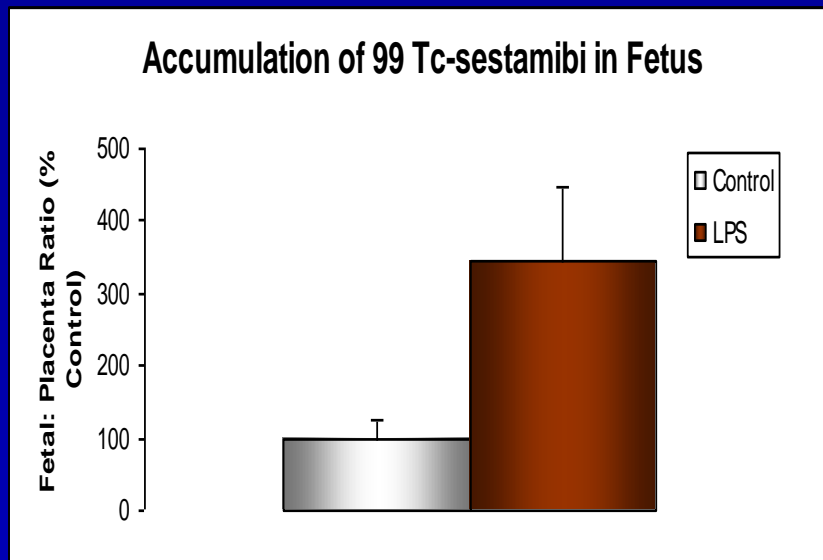


mRNA expression 12-18 hr post LPS (1 mg/kg) on GD17

↑ Fetal Accumulation of Substrates in Endotoxin-treated rats

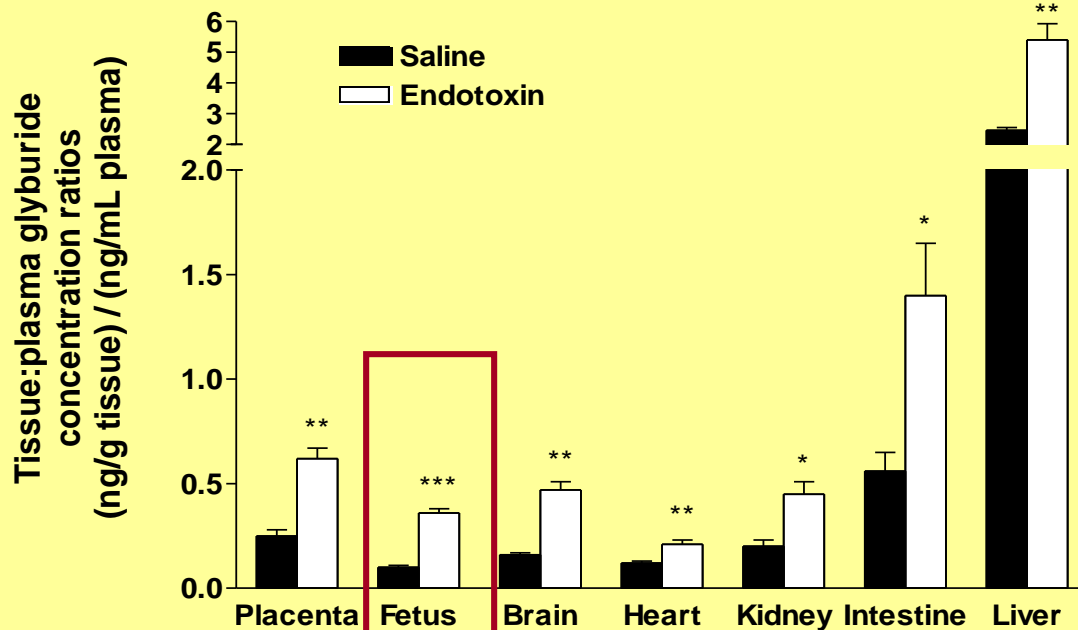
↑ Fetal Accumulation
of PGP (ABCB1)
Substrate-
⁹⁹Tc-sestamibi

J Nucl Med. 2005 ;46 :1537-45



↑ Fetal Accumulation
of BCRP (ABCG2)
Substrate- Glyburide

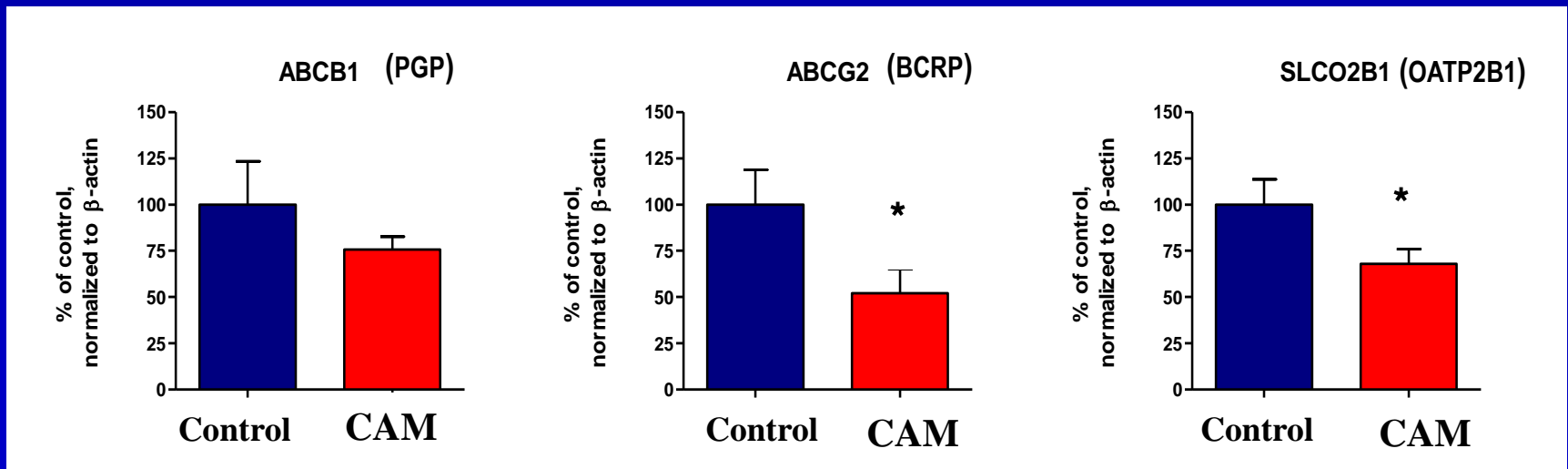
DMD. 2008;36:1944-50



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.

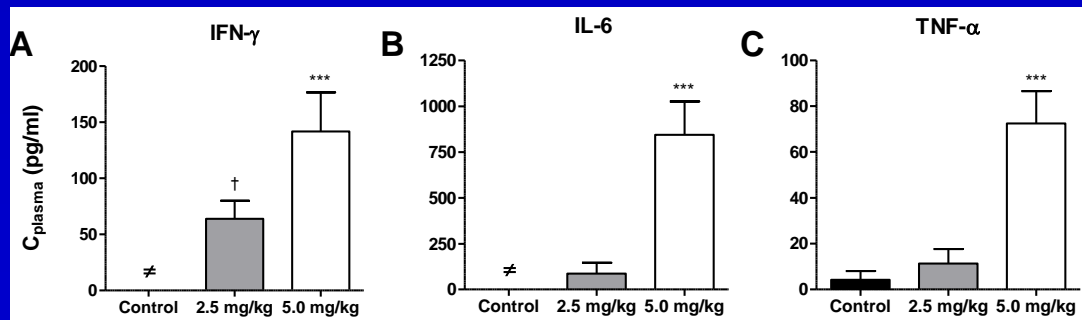
Results from western blot immuno-detection (mean \pm SEM, n=14/group).

↓ Expression of important drug / steroid transporters

**OTHER MODELS OF INFECTION
&
INFLAMMATION**

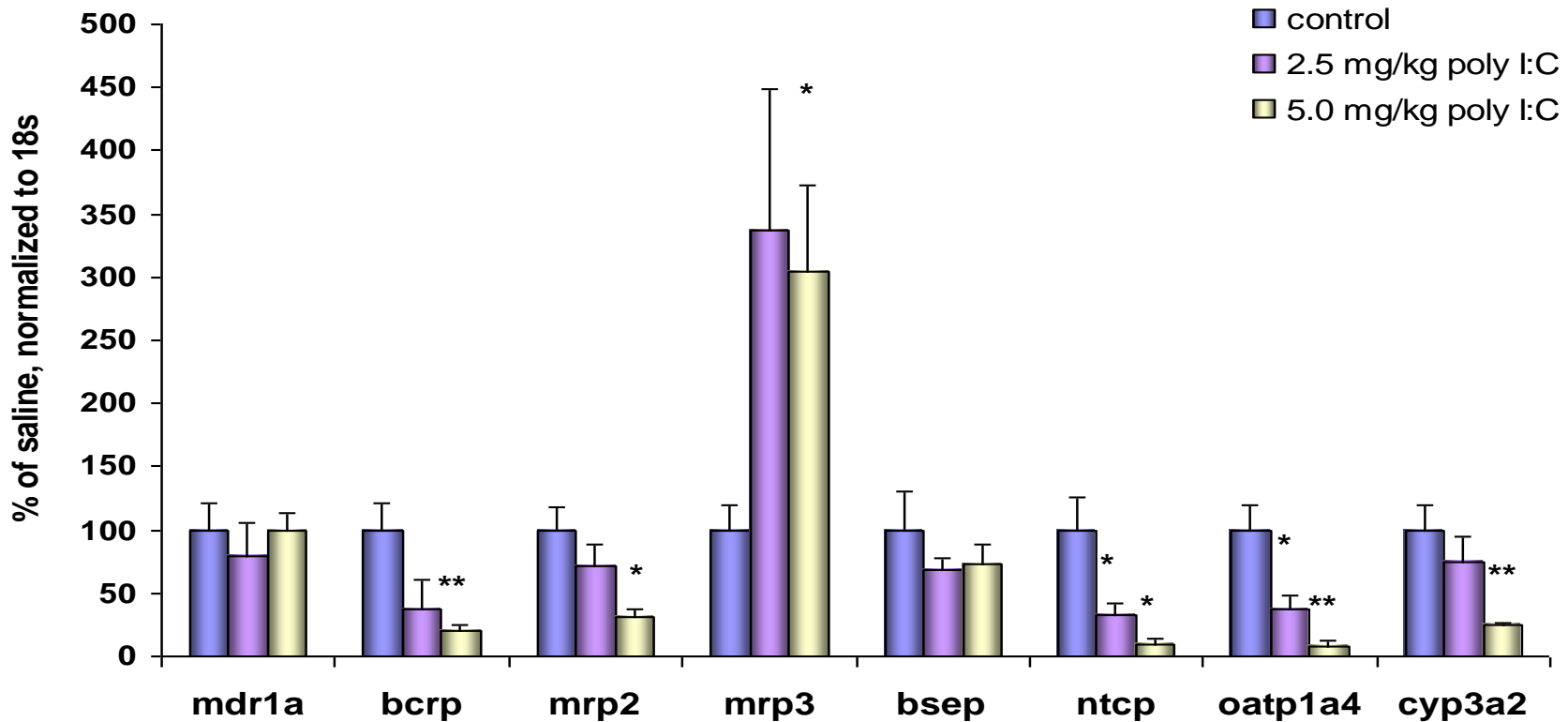
Viral Infections

- ➔ 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.



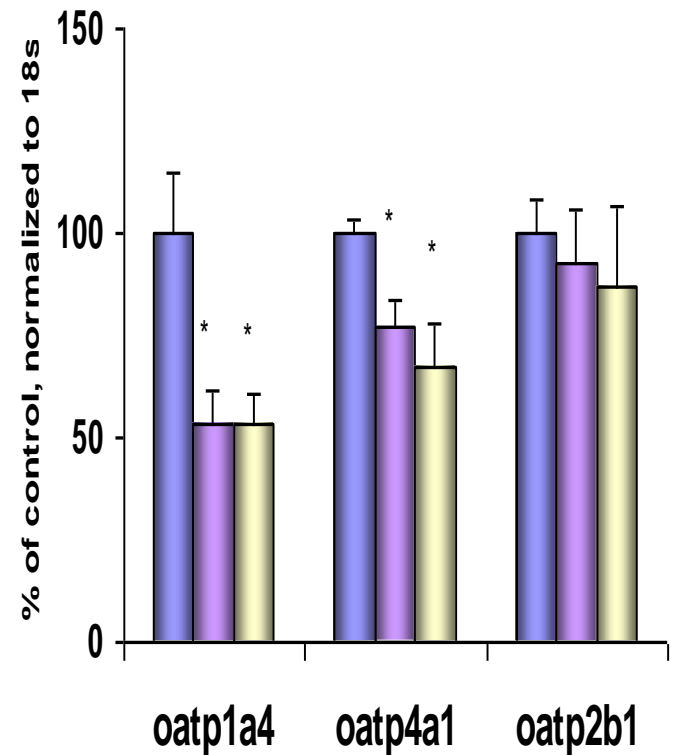
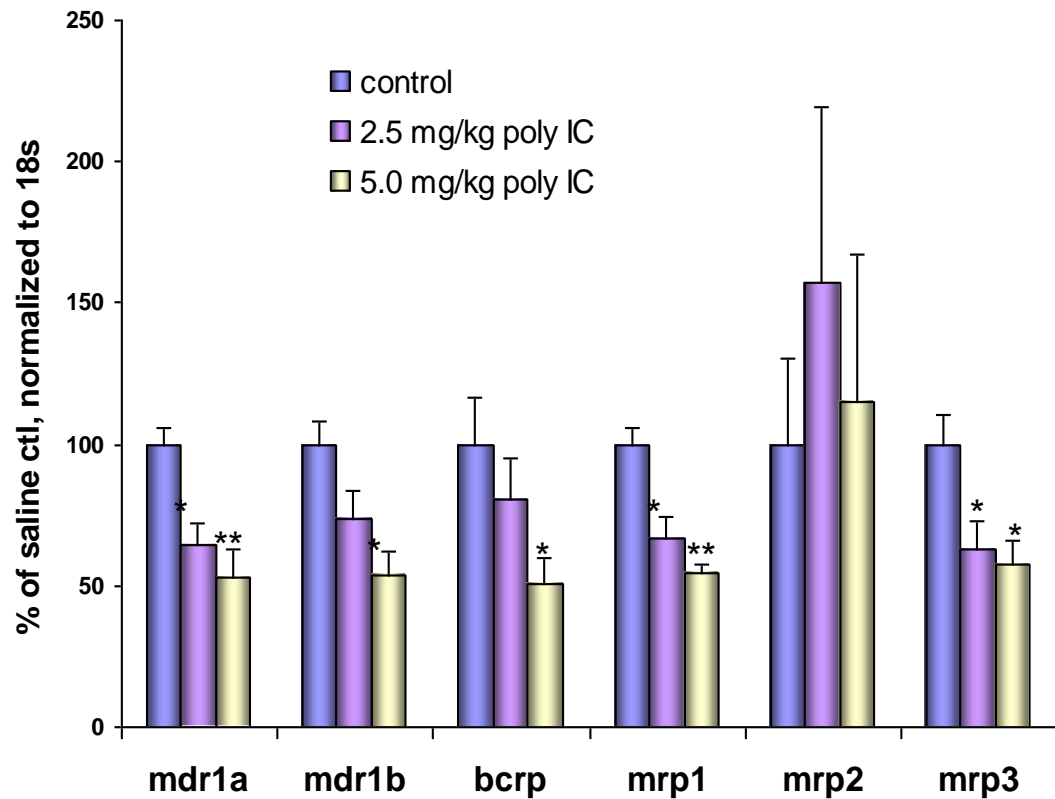
Impact of Poly I:C on Hepatic Expression of Transporters in Pregnant Rats.

Effect of poly I:C on hepatic transporters



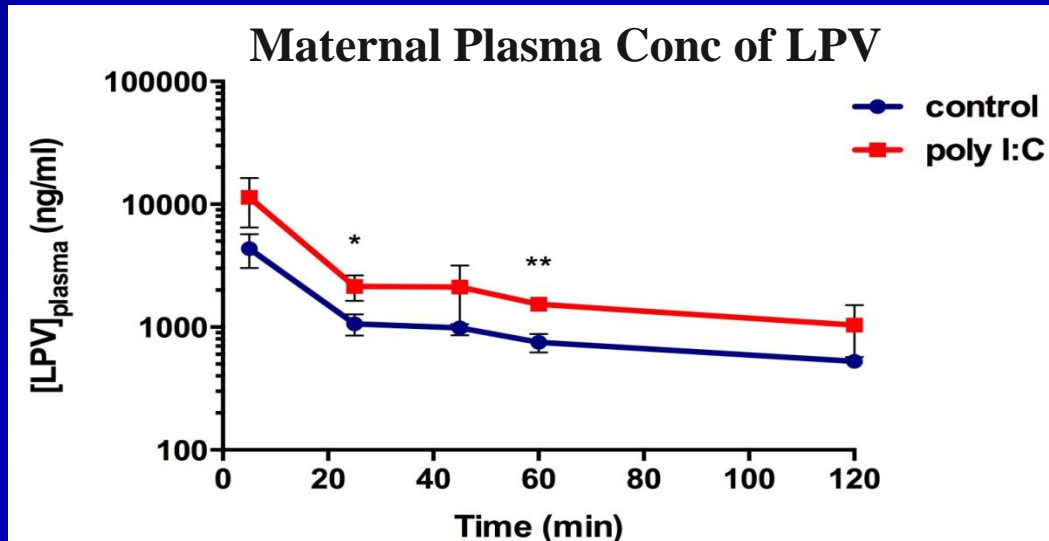
mRNA expression 24 hr post Poly I:C

Decreased Expression of many ABC and SLC Transporters in Placentas of Poly I:C - treated Rats

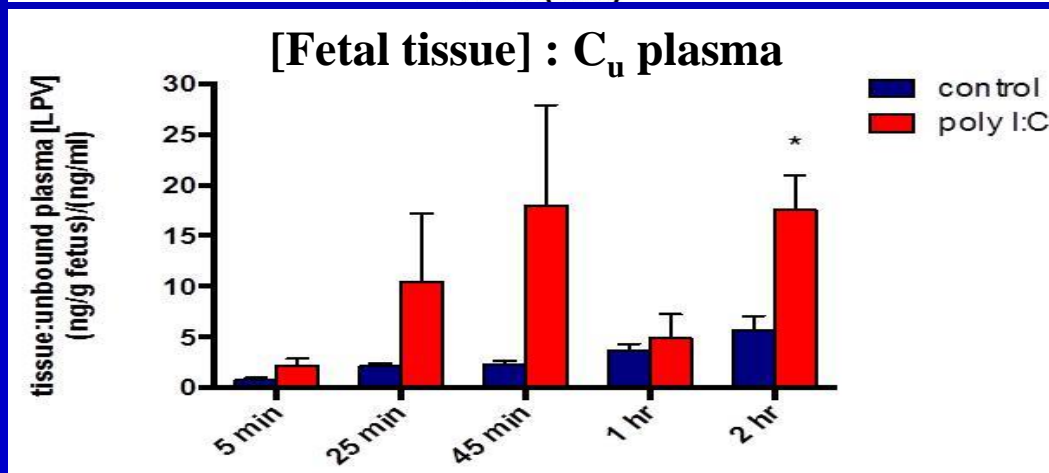


mRNA expression 24 hr post Poly I:C

Impact on Maternal and Fetal levels of the anti-HIV protease inhibitor- Lopinavir (LPV)



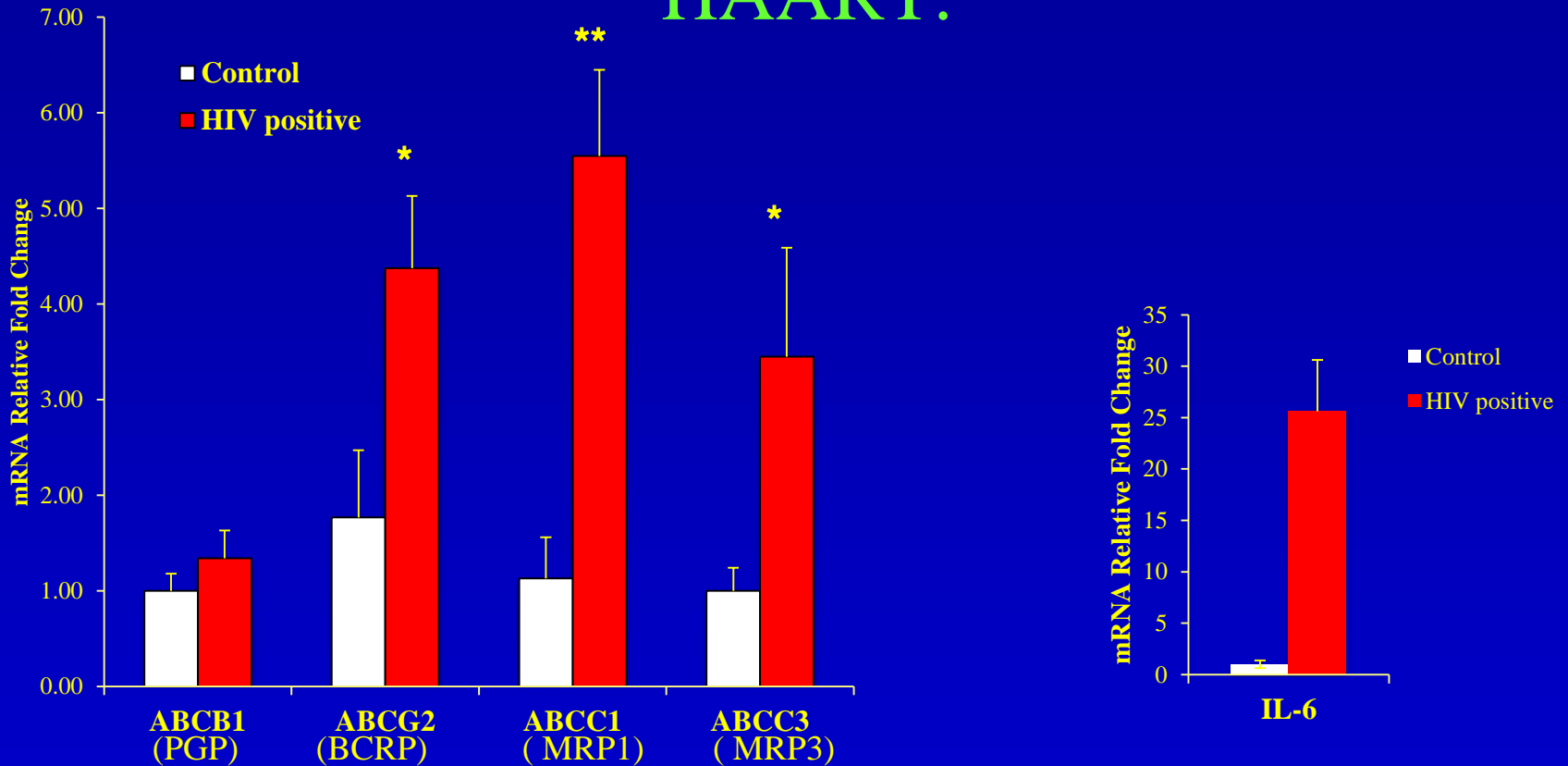
↑ AUC
↓ Clearance
↓ fu
↑ Vd



↑ Accumulation
↓ Pgp expression

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.

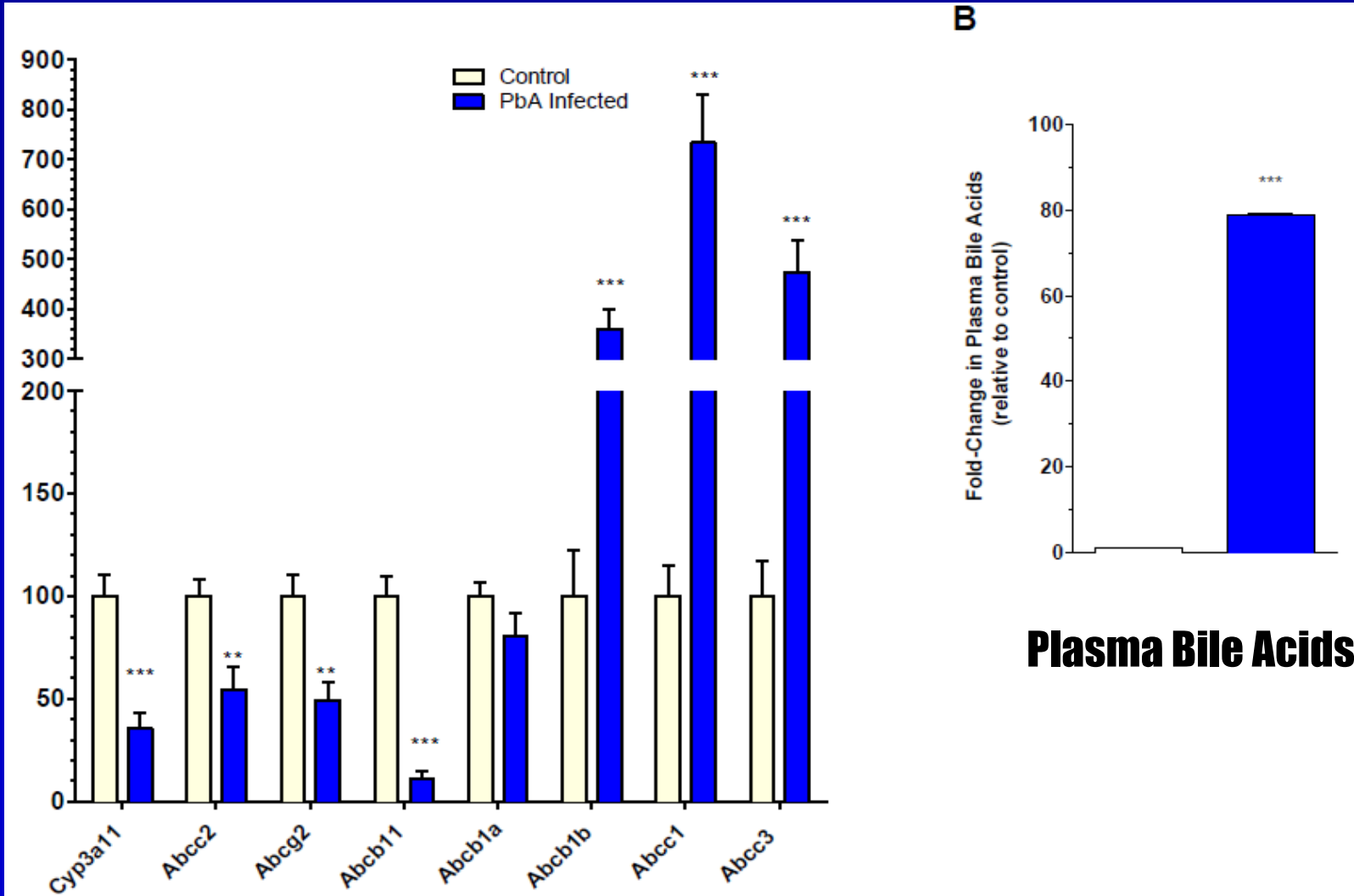
➡ Animal model: (Dr. Kevin Kain – University of Toronto)

➤ *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

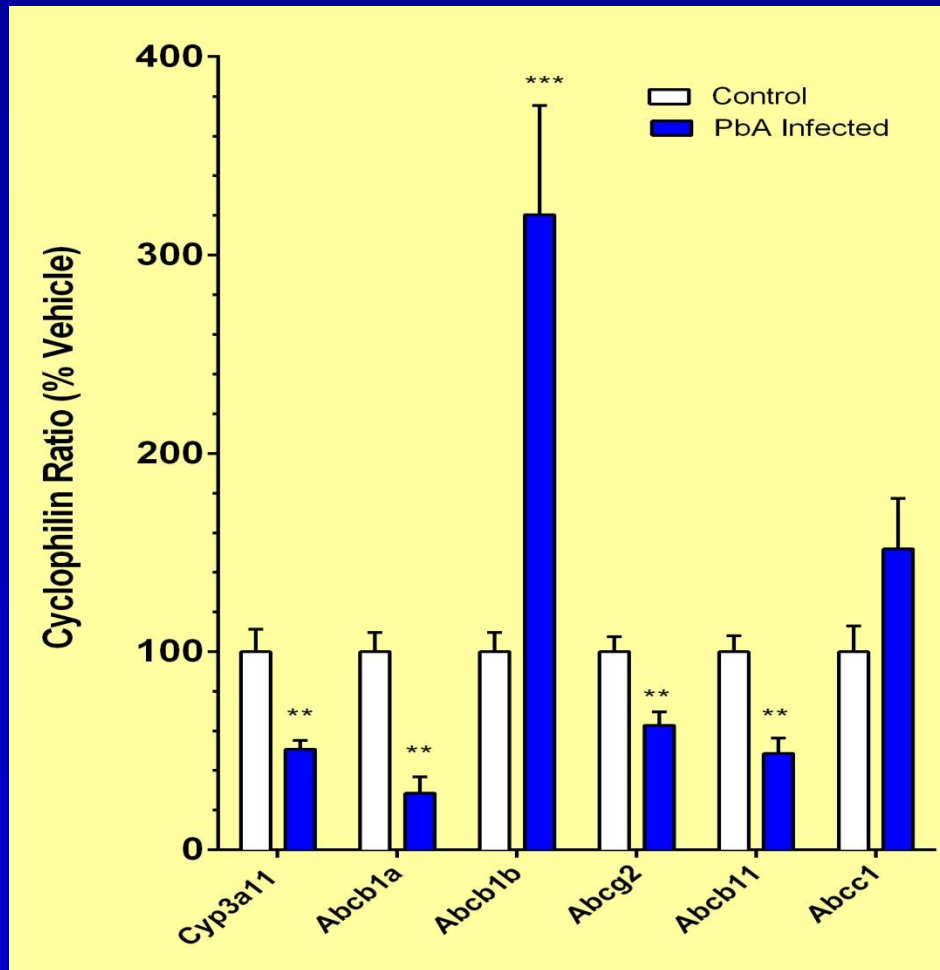
Impact of Malaria on Expression of Transporters in Maternal Liver



Mrp2 Bcrp Bsep

Mdr1b Mrp2 Mrp3

Impact of Malaria (*P. Berghei*) Infection on Expression of Transporters in Fetal Liver



Mdr1a Mdr1b Bcrp Bsep

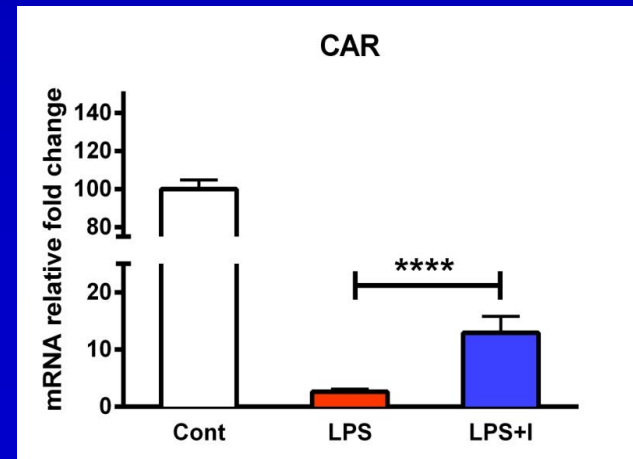
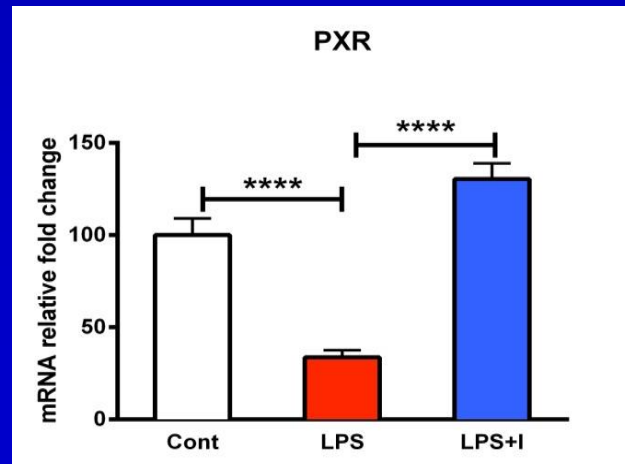
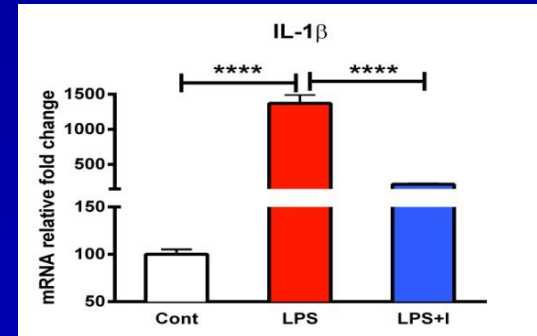
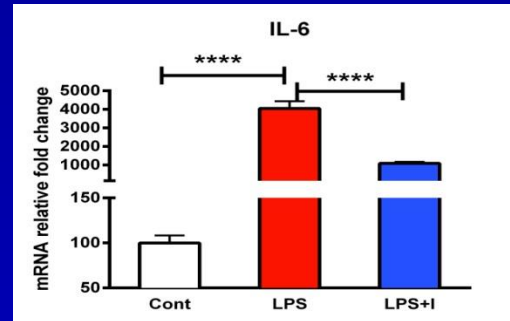
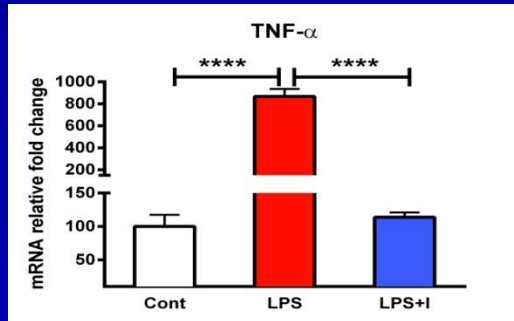
Downregulation of Bcrp, Bsep, Cyp3A & Upregulation of mdr1b in fetal liver similar to that seen in Maternal liver.

MECHANISMS?

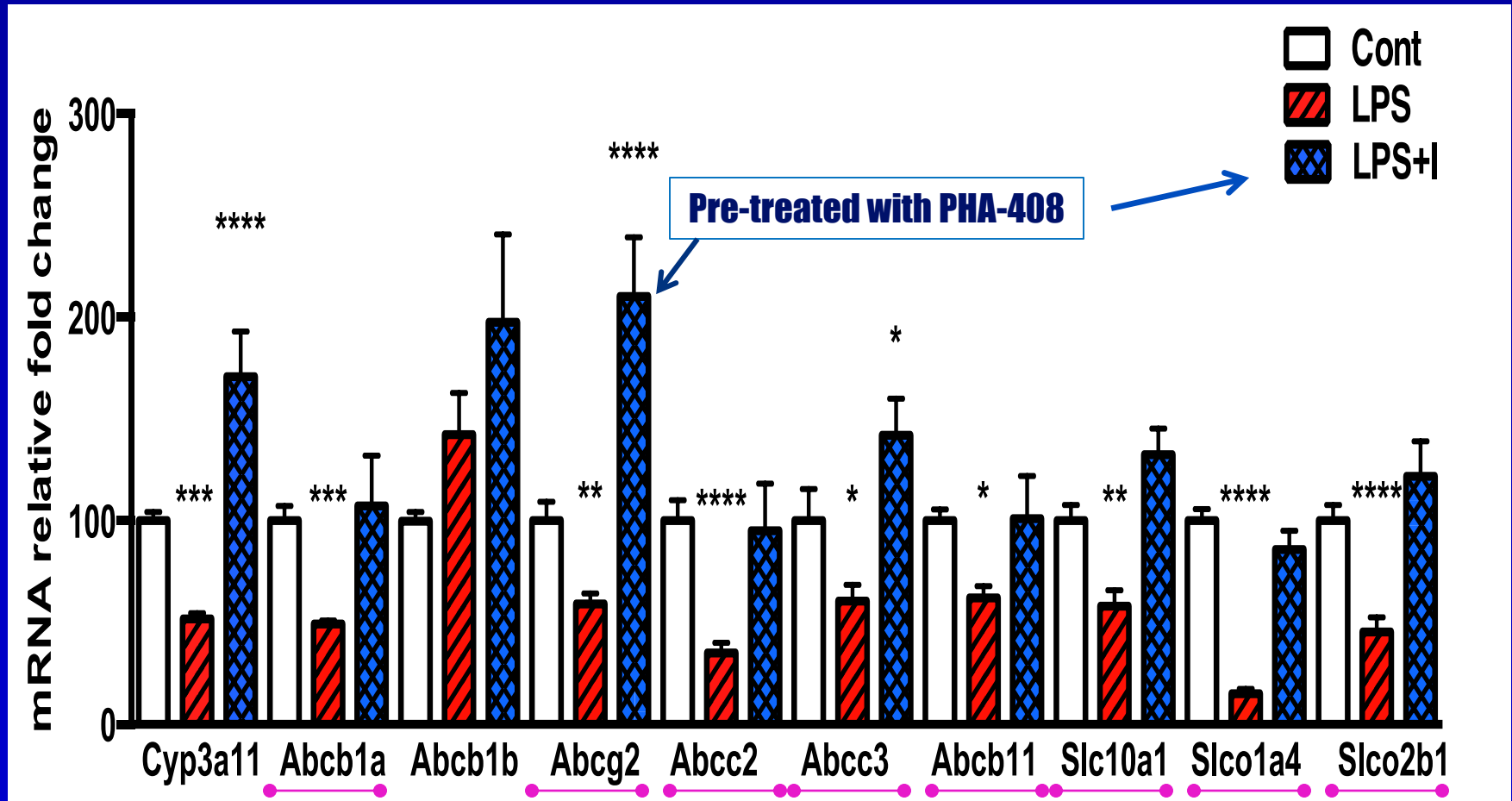
Involvement of Nuclear Factor Kappa B (NF- κ B)

- 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

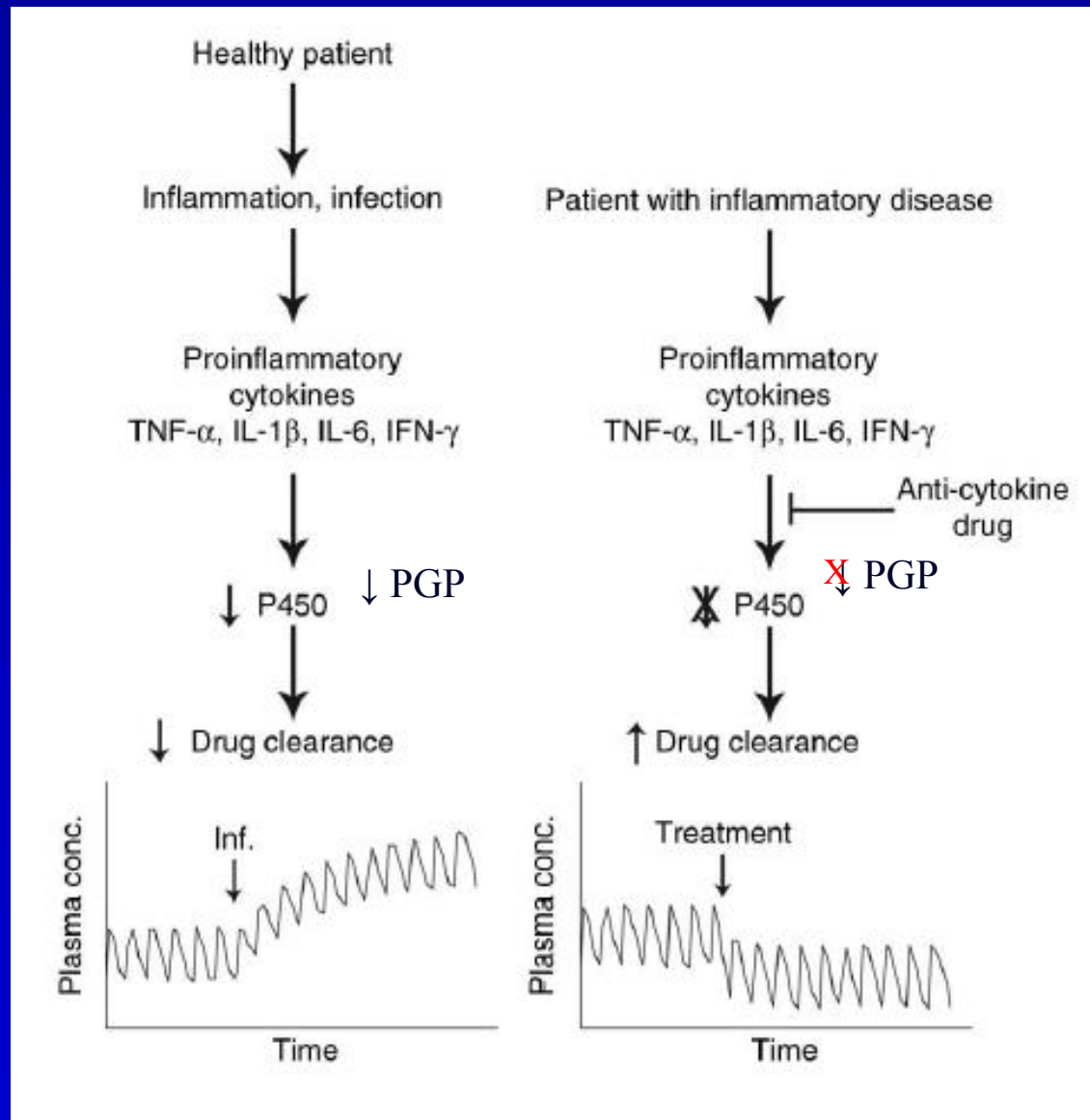
In vivo administration of selective NF- κ B inhibitor: PHA-408 (inhibits I κ B kinase), suppressed cytokine induction and PXR downregulation in endotoxin (LPS) -treated mice



Inhibition of NF- κ B suppresses downregulation of Cyp3A & ABC- efflux and SLC-uptake transporters in endotoxin-treated mice



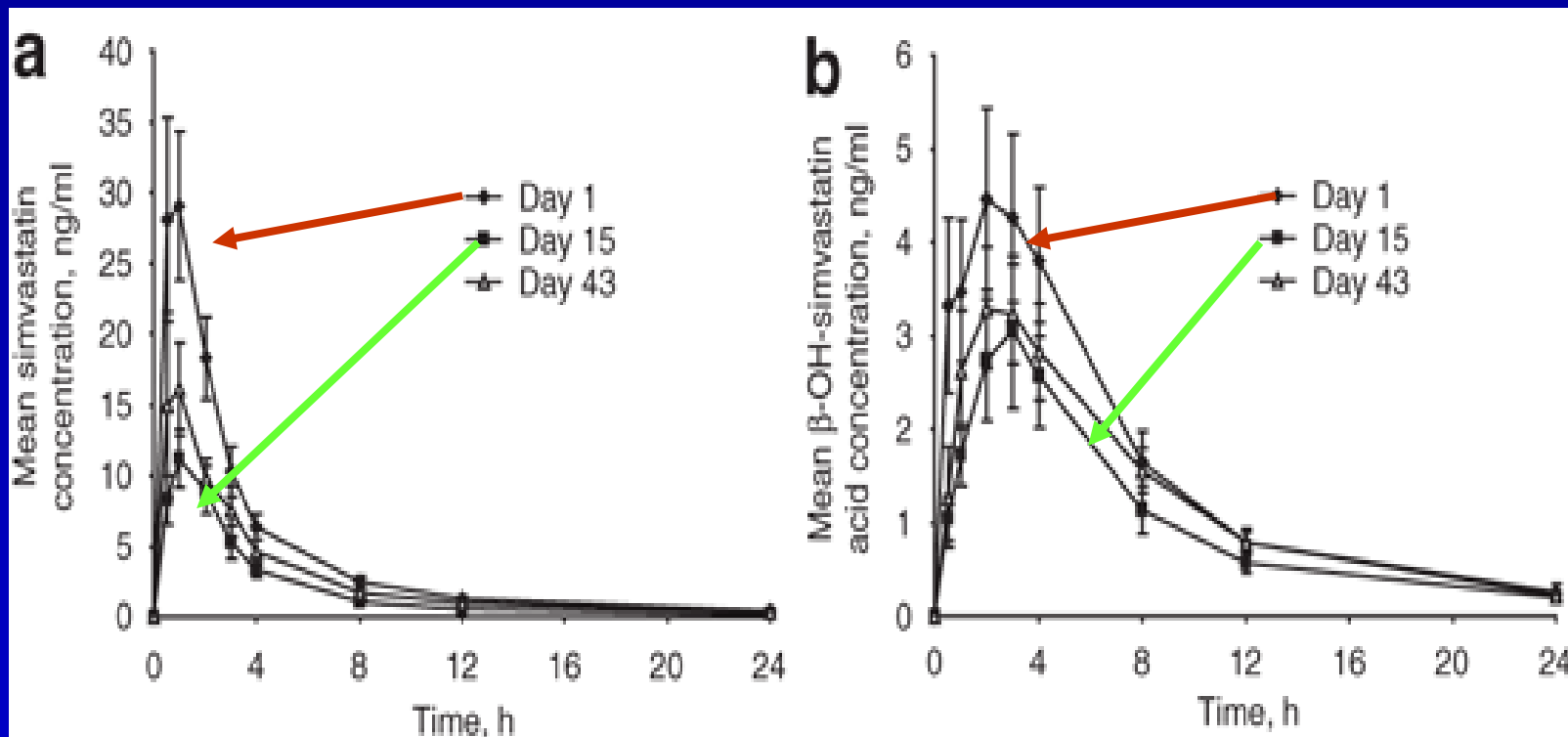
What if the Inflammatory response is controlled or decreased?



Intervention

Clinical Disease-drug-drug interaction

Tocilizumab (IL-6R antibody) and Simvastatin (CYP3A substrate) in patients with rheumatoid arthritis.

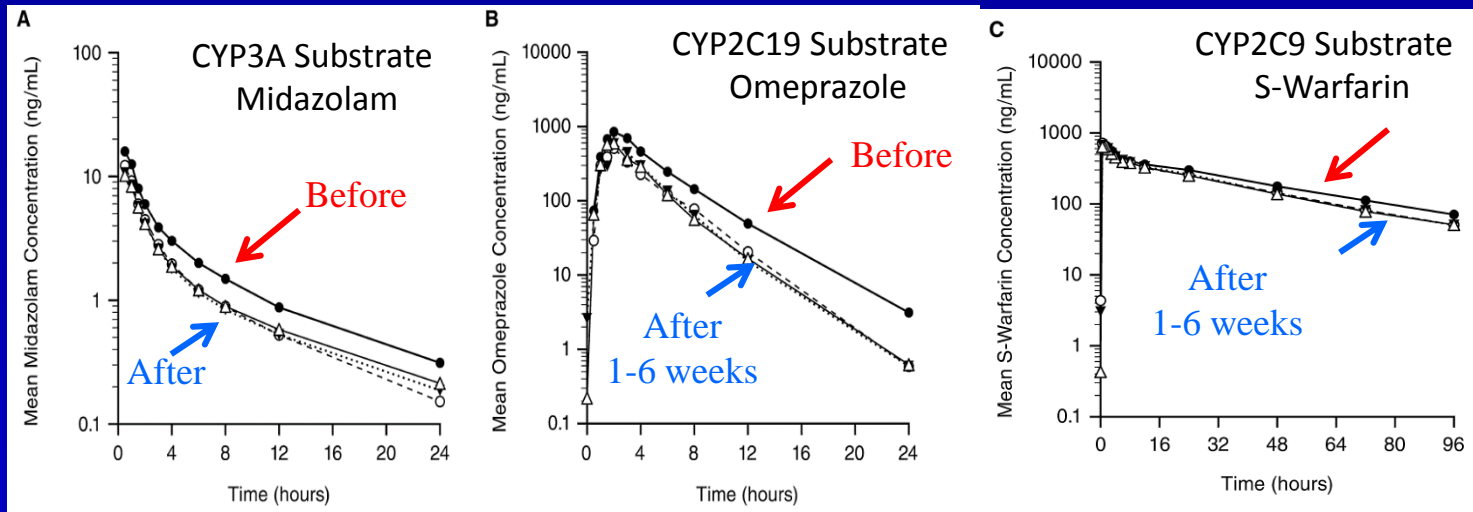


Exposure to Simvastatin and CRP levels significantly reduced after IL-6R antibody (10 mg/kg Day 8) in RA patients (n=12)

Attributed to IL-6 mediated changes in CYP3A activity

Disease- DDI with **Sirukumab (IL-6 mAb)** in patients with active Rheumatoid Arthritis

Zhuang et al. J Clin Pharmacol. 2015



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- κ B 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:ddl interactions

Acknowledgement

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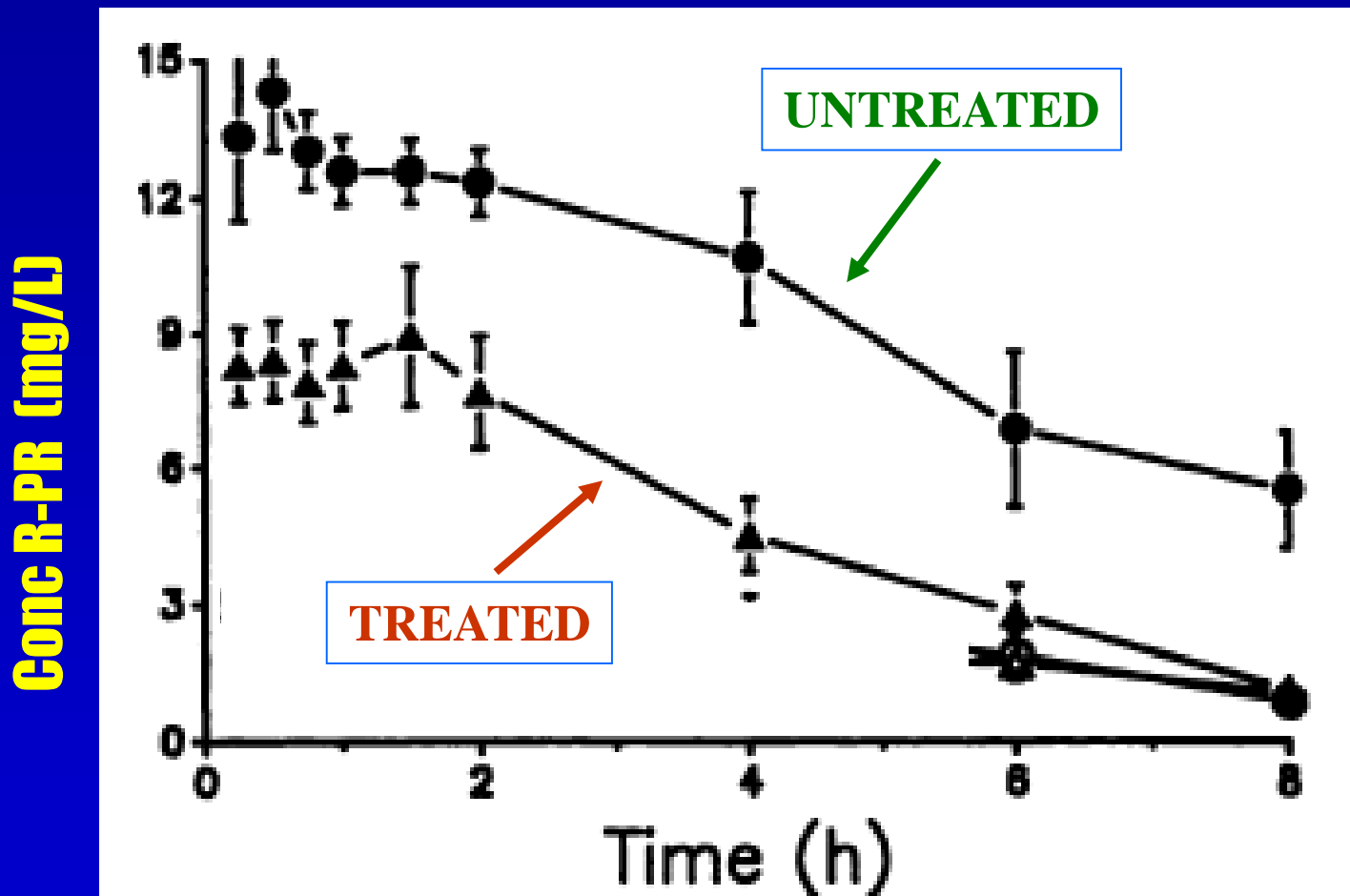
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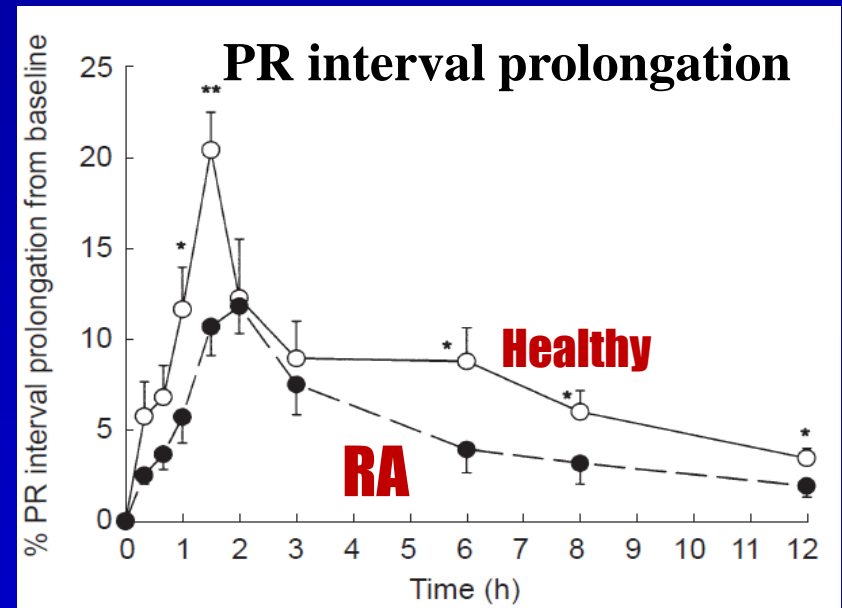
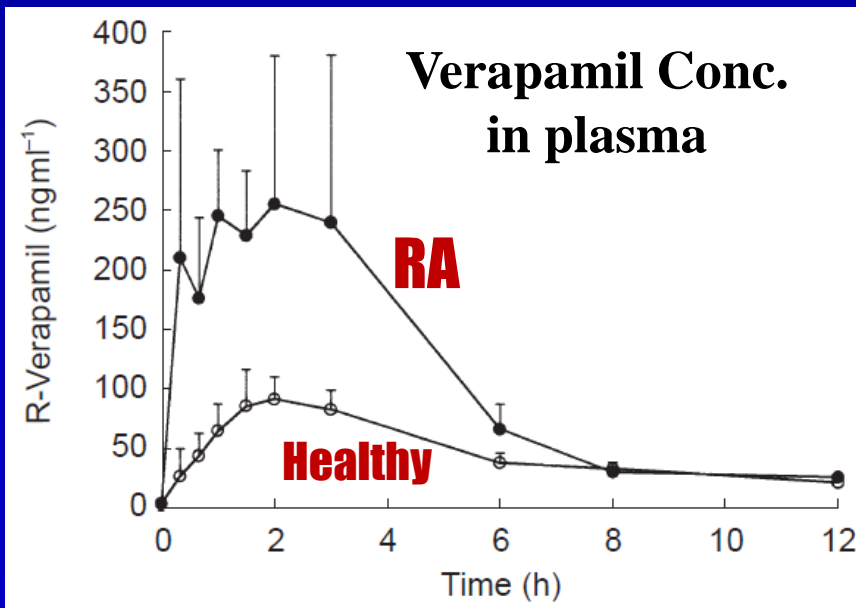
Canadian CIHR Operating Grants
Rx &D HRF- CIHR Research Career Award
Connaught – University of Toronto

Plasma Levels of Propranolol in Ketoprofen-Treated and Untreated Arthritic Rats



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.**