SYSTEMS PHARMACOLOGY MODELING PREDICTS HEPATOTOXIC POTENTIAL OF TROGLITAZONE AND PIOGLITAZONE

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Abstract

Troglitazone (TGZ) caused life-threatening drug-induced liver injury (DILI) in diabetic patients, whereas the next in class, pioglitazone (PGZ), has rarely been associated with DILI. Inhibition of bile acid transport, which may result in accumulation of toxic bile acids in hepatocytes, is one proposed mechanism of TGZ-mediated hepatotoxicity. However, PGZ is a more potent inhibitor of the bile salt export protein (BSEP) than TGZ based on in vitro membrane vesicle transport studies. In the current study, the hepatotoxic potential of TGZ and PGZ due to interference with bile acid homeostasis was investigated using DILIsym®, a systems pharmacology model of DILI. Experimentally measured inhibition constants of TGZ, TGZ-sulfate, and PGZ for multiple bile acid transport proteins were employed to simulate the altered bile acid disposition and subsequent DILI in humans. In the virtual human population (Simpops™), administration of 200–600mg/day TGZ for 6 months resulted in delayed increases in serum alanine transaminase (ALT) > 3X upper limit of normal (ULN) in 0.3–5.1% of the population, with concomitant elevations in serum bilirubin > 2X ULN in 0.9–3.6% of the population. The simulated time to peak ALT was 116±60 days. These results were similar to observations from the clinical trials where 200–600mg/day TGZ elicited serum ALT elevations > 3X ULN in 1.9% of treated patients with time to peak ALT of 147±86 days. No hepatotoxicity was predicted in the Simpops™ after administration of clinically relevant doses of PGZ (15-45mg/day) for 6 months, consistent with the clinical observations. In summary, mechanistic modeling based only on bile acid homeostasis adequately predicted the incidence and delayed presentation of TGZ hepatotoxicity, and correctly predicted relative liver safety of PGZ. These results demonstrate the utility of systems pharmacology models that integrate physiology and experimental data to evaluate DILI mechanisms and identify potential risk factors for DILI. Importantly, these mechanistic models may be useful to prospectively predict the hepatotoxic potential of new drug candidates.

Objective

- Assess the hepatotoxic potential of troglitazone (TGZ) and pioglitazone (PGZ) due to interference with bile acid homeostasis using DILIsym®, a systems pharmacology model of drug-induced liver injury.

Introduction

- Drug-induced liver injury (DILI) is one of the primary reasons for the failure of pharmaceutical agents during drug development as well as withdrawal of approved drugs from the market.[1]
- Troglitazone (TGZ) caused delayed and life-threatening DILI and was withdrawn from worldwide markets. TGZ and its major metabolite, TGZ sulfate (TS), are potent inhibitors of multiple bile acid transporters, which may lead to accumulation of toxic bile acids in hepatocytes and hepatotoxicity. [2,3,4]
- Pioglitazone (PGZ) also inhibits bile acid transporters, but has rarely been associated with DILI.[5,6]
- DILIsym® is a systems pharmacology model of DILI that incorporates drug/metabolite disposition, bile acid physiology and pathophysiology, the hepatocyte life cycle, and liver injury biomarkers.[7,8]

Results

Bile Acid-Mediated Hepatotoxicity

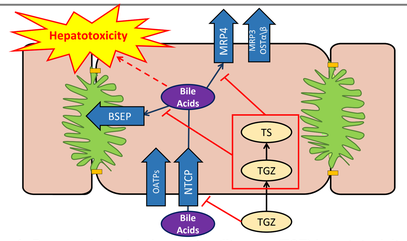


Figure 1. Proposed mechanism of troglitazone (TGZ) hepatotoxicity. TGZ and its major metabolite, TGZ sulfate (TS), are potent inhibitors of hepatic bile acid transporters, which might lead to hepatic bile acid accumulation and subsequent toxicity.[2,3,4] BSEP, bile salt export pump; NTCP, sodium-taurocholate cotransporting polypeptide; OATP, organic anion transporting polypeptide; MRP, multidrug resistance-associated protein' OST, organic solute transporter.

Bile Acid Inhibition Model Diagram

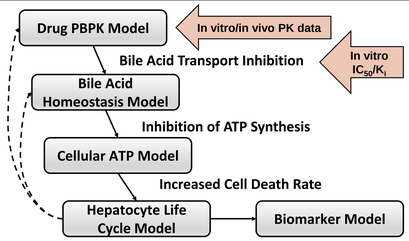


Figure 2. Scheme depicting the bile acid transport inhibition module in DILIsym®. Bile acid homeostasis model was constructed previously.[8] Drug-mediated hepatic accumulation of bile acids inhibits ATP synthesis. Depletion of hepatic ATP leads to necrotic cell death and elevations in serum biomarkers of hepatocellular injury and function (e.g., ALT, AST, bilirubin).[7,8]

PBPK Simulation Results

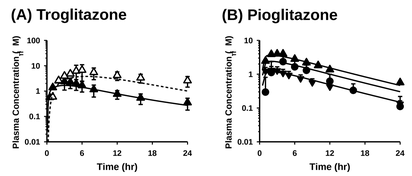


Figure 3. Predicted and observed plasma concentration of troglitazone (TGZ), TGZ sulfate (TS), and pioglitazone (PGZ). (A) Closed and open triangles represent observed plasma concentrations of TGZ and TS, respectively, in humans administered 400 mg oral TGZ. Solid and dashed lines represent simulated plasma TGZ and TS concentrations, respectively. (B) Closed symbols represent observed plasma concentrations of pioglitazone at the oral doses of 15 mg (inverted triangle), 30 mg (circle), and 45 mg (triangle). Solid lines represent simulated plasma PGZ concentrations.

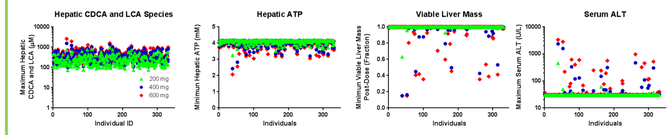
Hepatotoxicity Simulation Results

Table 1. Summary of troglitazone (TGZ) and pioglitazone (PGZ)-mediated hepatotoxicity in simulated human populations (SimPops™) and clinical trials.

	Troglitazone					Pioglitazone	
	Simulations[a]			Clinical Trials		Simulations[a]	Clinical Trials
	200 mg (n=331)	400 mg (n=331)	600 mg (n=331)	200–600 mg (n=2510)	Placebo (n=475)	15-45 mg (n=331)	15-45 mg (n=3650)
ALT > 3X ULN (%)	0.3	3.0	5.1	1.9	0.6	0	0.33
ALT > 5X ULN (%)	0.3	1.8	4.2	1.7	N/A	0	0.25
ALT > 8X ULN (%)	0.3	1.8	3.6	0.9	0.0	0	0.03
ALT > 30X ULN (%)	0	0.6	0.9	0.2	0.0	0	0
Time to peak ALT (Days)[b]	180[c]	118 ± 61	111 ± 61	147 ± 86	N/A	N/A	N/A
Total Bilirubin > 2X (%)	0.3	1.8	3.6	N/A	N/A	0	N/A
Hy's Law cases (%)	0.3	1.8	3.6	N/A	N/A	0	N/A

[a] Each dose level was simulated for 6 months; [b] Mean ± S.D; [c] S.D. was not calculated because only one individual showed ALT elevation > 3X ULN; ALT, alanine aminotransferase; ULN, upper limit of normal; N/A, not available.

(A) Troglitazone



(B) Pioglitazone

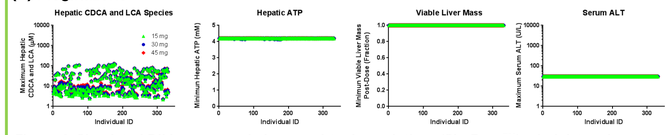


Figure 4. Simulated DILI responses in human virtual populations (SimPops™) administered a specified dose of troglitazone (A) or pioglitazone (B). Predicted maximum hepatic accumulation of toxic bile acids [chenodeoxycholic acid (CDCA) and lithocholic acid (LCA) species] and DILI responses (i.e., minimum hepatic ATP, minimum viable liver mass, maximum serum ALT) post-dose in human SimPops™ at oral doses of 200 (green triangle), 400 (blue circle), or 600 (red diamond) mg/day troglitazone for 6 months (A), and at oral doses of 15 (green triangle), 30 (blue circle), or 45 (red diamond) mg/day pioglitazone for 6 months (B).

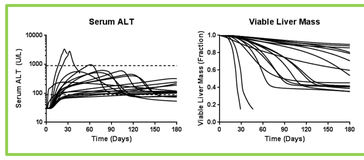
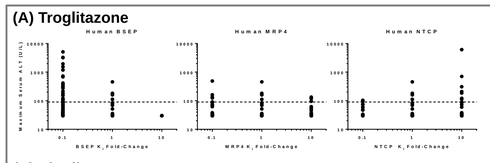


Figure 5. Simulated time course of serum ALT and viable liver mass in individuals susceptible to troglitazone (TGZ) hepatotoxicity. In human SimPops™ administered 600 mg/day TGZ for 6 months, individuals with serum ALT elevations > 3X ULN (n=17) are presented. Two individuals lost >85% of viable liver mass and was classified as dead.

At common clinical doses of troglitazone (200–600mg/day), the simulated incidence rates of serum ALT elevation were similar to those observed in clinical trials. The delayed ALT peak was recapitulated by the simulation; in the current model, delayed ALT elevations were driven by a delayed accumulation of bile acids in hepatocytes. No hepatotoxicity was predicted at clinical doses of pioglitazone (15–45mg/day) due mainly to the low hepatic exposure of pioglitazone as a result of extensive hepatic metabolism.

Sensitivity Analysis

(A) Troglitazone



(B) Pioglitazone

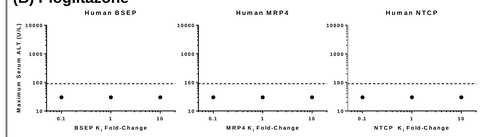


Figure 6. Sensitivity analysis of transporter inhibition constants. Inhibition constants for BSEP, MRP4, and NTCP were altered 10-fold in either direction of the values measured in isolated transport systems. Predicted maximum serum ALT concentrations in human SimPops™ after an oral dose of 600mg/day troglitazone (A) and 45 mg/day pioglitazone (B), respectively, for 1 month are presented. Dashed lines represent 3X baseline ALT (90 U/L).

Troglitazone hepatotoxicity is sensitive to transporter inhibition constants. Pioglitazone is not likely to exert bile acid-mediated hepatotoxicity in humans.

Troglitazone Multiple Regression Analysis

Table 2. List of parameters varied in the human SimPops™ and results of multiple regression analysis in human SimPops™ administered 600 mg/day troglitazone (TGZ) for 6 months.

Parameter Name	Parameter Description	Significance	Standardized Coefficient[a]
Bile Acid Homeostasis Sub-model			
LCA-sulfate uptake V_{max}	Maximum velocity of hepatic uptake of LCA-sulfate	N/S	-0.08
LCA-sulfate canalicular efflux V_{max}	Maximum velocity of biliary excretion of LCA-sulfate	P < 0.001	0.41
CDCA-amide uptake V_{max}	Maximum velocity of hepatic uptake of CDCA-amide	N/S	-0.01
CDCA-amide canalicular efflux V_{max}	Maximum velocity of biliary excretion of CDCA-amide	P < 0.01	0.14
CDCA-amide basolateral efflux V_{max}	Maximum velocity of hepatic basolateral efflux of CDCA-amide	N/S	0.08
CDCA amidation V_{max}	Maximum velocity of CDCA amidation in hepatocytes	N/S	0.06
LCA-amide sulfation V_{max}	Maximum velocity of LCA-amide sulfation in hepatocytes	N/S	-0.06
LCA synthesis V_{max}	Maximum velocity of LCA synthesis by the gut microbiome	P < 0.001	-0.21
Uptake regulation scaling factor	Scaling factor governing the magnitude of feedback regulation of hepatic uptake transporter function by hepatic bile acid accumulation	N/S	0.02
Canalicular efflux regulation scaling factor	Scaling factor governing the magnitude of FXR-mediated feedback regulation of hepatic canalicular transporter function by hepatic bile acid accumulation	P < 0.001	0.2
Drug PBPK Sub-model			
TGZ intestinal absorption K_{ab}	First-order rate constant for TGZ absorption from intestine	N/S	-0.05
TGZ hepatic uptake V_{max}	Maximum velocity of TGZ hepatic uptake	N/S	-0.07
TGZ sulfation V_{max}	Formation rate of TGZ-sulfate (TS)	N/S	-0.06
TS biliary clearance	Biliary clearance of TS	P < 0.001	0.15
Other System-Specific Parameters			
Body weight[c]	Body weight	P < 0.001	0.15
Toxicity K_m for CDCA and LCA species[c]	Intracellular bile acid concentrations that induce half-maximal inhibition of ATP synthesis	P < 0.001	0.15

[a] Parameter estimates that would have resulted from the regression if all of the variables had been standardized to a mean of 0 and a variance of 1. The greater the absolute value of the standardized coefficient, the greater the effects of the independent variable on the model output; LCA, lithocholic acid; CDCA, chenodeoxycholic acid; FXR, farnesoid X receptor; N/S, not significant.

Decreased function of hepatic canalicular transporters that mediate excretion of bile acids and troglitazone sulfate, increased LCA synthesis in the intestinal lumen, decreased feedback regulation of bile acid transporters, smaller body weight, and decreased mitochondrial function are potential risk factors for troglitazone-mediated hepatotoxicity.

Methods

Physiologically-based pharmacokinetic (PBPK) model development PBPK models of troglitazone (TGZ) and pioglitazone (PGZ) were developed using in vitro and in vivo pharmacokinetic data available from the literature (see reference 4 for details).

Construction of human virtual population (SimPops™) Human population samples (n=331) with variability in 10 parameters in the bile acid homeostasis sub-model were constructed previously within DILIsym®.[8] Parameters governing drug disposition, body weight, and sensitivity of hepatic ATP decline to hepatic bile acid accumulation also were varied using the probability distribution of each parameter obtained from the literature.

Simulation of DILI responses Perturbation of bile acid disposition and DILI responses after TGZ (200, 400, or 600mg/day) or PGZ (15, 30, or 45mg/day) administration for 6 months in human SimPops™ were simulated using PBPK model predictions of TGZ/TS or PGZ disposition, a previously developed bile acid homeostasis sub-model[8], and bile acid transport inhibition constants for TGZ/TS or PGZ (i.e., K_i, IC_{50}) measured in isolated membrane vesicle transport systems.

K_i/IC_{50} (μM)	TGZ	TS	PGZ
BSEP	1.3	0.23	0.5
MRP4	21[a]	8[b]	49.5
NTCP	0.33[a]	0.33[a]	4.04

[a] IC_{50} (Ki otherwise)
[b] Noncompetitive inhibition (competitive otherwise)

Sensitivity Analysis To assess the sensitivity of DILI responses to inhibition constants, simulations were performed with 10-fold smaller or greater inhibition constants of TGZ/TS or PGZ for BSEP, MRP4, and NTCP (600mg/day TGZ or 45mg/day PGZ for 1 month).

Multiple Regression Analysis To identify the most important parameters in the context of bile acid-mediated DILI in humans administered TGZ, a multiple regression analysis was performed with minimum hepatic ATP as the dependent variable. Sixteen parameters used to develop the human SimPops™ were utilized as independent variables. Because the units of independent variables were different by orders of magnitude, standardized coefficients were calculated to determine which of the independent variables have a greater effect on the minimum hepatic ATP. Statistical analyses were performed using JMP 10 (SAS, Cary, NC).

Conclusion

- Mechanistic modeling based on bile acid effects adequately predicted the incidence and delayed presentation of troglitazone hepatotoxicity, and the relative liver safety of pioglitazone.
- Systems pharmacology models integrating physiology and experimental data can evaluate DILI mechanisms and may be useful to predict hepatotoxic potential of drug candidates.

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Acknowledgements

The current research was supported by members of the DILI-sim Initiative and NIH R01GM41935

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