Troglitazone Metabolism and the Impact of its Metabolites on Taurocholic Acid Transport in the Hurelflux™ Primary Human Hepatic Co-culture System

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ABSTRACT

PURPOSE:
To examine the effects of troglitazone metabolites on taurocholic acid transport in the Hurelflux™ primary human hepatic co-culture system.

METHODS:
The Hurelflux™ primary human hepatic co-culture model using hepatocytes from a single donor were assessed in a 24-well format. Hepatocytes were co-treated or pretreated (2 h and 72 h) with 10 µM troglitazone (TGZ) followed by addition of TCA (5 µM) for 20 mins before the medium, cellular and bile contents were collected. Control experiments were conducted with DMSO as the mock treatment instead of troglitazone. Samples were analyzed in negative ionization mode on an AB Sciex Qtrap 5500 using the following MRM transitions: TCA (514.2 → 79.8), TGZ (440.1 → 397.0), troglitazone sulfate (TGZ-SO_3; 520.1 → 440.1), troglitazone quinone (TGZ-Q; 456.1 → 413.1), and troglitazone glucorononide (TGZ-Gluc; 616.1 → 440.1). Analytes were separated using a Kinetex XB-C18 column (50 x2.1 mm; 1.7 µm) and a reverse-phase LC gradient over 5.5 minutes. Mobile phases consisted of water with 10 mM ammonium acetate and acetonitrile. The TCA biliary excretion index (BEI), intrinsic biliary clearance ($Cl_{int,biliary}$), and uptake kinetics were calculated for the control and the TGZ treatment studies.

RESULTS:
Troglitazone was rapidly metabolized and was not detected in medium, bile, or cell samples after 20 minutes. Troglitazone sulfate was the major metabolite detected in all matrices. Lower levels of TGZ-Q were detectable only in the medium, while the glucuronide was observed in the media, bile, and the cells. The BEI and intrinsic biliary clearance of TCA after the 20 minute incubation with no pre-treatment were 65% and 28 µL/min/mg protein, respectively, and were consistent with reported values from Hurel (66% ± 9; 41%-72%[1,2] and 23 ± 3 µL/min/mg protein). Troglitazone treatment did not alter the BEI but decreased the intrinsic biliary clearance of TCA by approximately 5-fold, 8-fold, and 6-fold in the TGZ co-dosed, 2-hour TGZ pretreated, and 72-hour TGZ pretreated groups, respectively. In addition to the reduced intrinsic biliary clearance of TCA, TGZ treatment markedly decreased the TCA uptake into the hepatocytes by approximately 15-fold compared to the control.

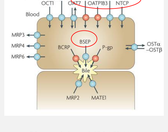
CONCLUSIONS:
The Hurelflux™ system demonstrated relevant metabolic activities by generating the reported human metabolites, primarily troglitazone sulfate (TGZ-SO_3). This model also showed transporter-mediated taurocholic acid clearance routes appear to be functioning in a manner similar to intact liver. Thus, this system can be utilized for assessing the modulation of transport by metabolites generated in situ. Studies are currently ongoing to elucidate the specific metabolite-transporter inhibition associated with the markedly reduced intrinsic biliary clearance and hepatocyte uptake of TCA.

INTRODUCTION

Troglitazone, a thiazolidinedione insulin sensitizer for the treatment of type-2 diabetes was removed from the market due to idiosyncratic liver toxicity. It and its major circulating metabolite troglitazone sulfate have been the focus of numerous studies examining the inhibition of transporters (BSEP, OATP1B1, OATP1B3, OATP2B1) involved in the disposition of taurocholic acid (Fig. 1). Cells transfected with a single transporter of interest and sandwich cultured hepatocytes (SCH) have been used in tandem to evaluate these complex transporter interactions using isolated and integrated approaches, respectively.

Hurel has developed a hepatocyte co-culture model that expresses drug metabolizing enzymes and transporters. Furthermore this system contains intact biliary canaliculi which allows the investigation of TCA or drug biliary excretion and clearance. In this study we used the Hurel model to further study the complex interactions of TGZ and TGZ-SO_3 on the disposition of endogenous TCA in the hepatocytes and exogenous d5-TCA added to the medium. The amount of TGZ, TGZ-SO_3, TCA and d5-TCA were simultaneously monitored in the cells, media, and bile in an effort to relate levels in these compartments with inhibition and directional flux.

Figure 1
Transporters Involved in TCA Disposition



Giacomini et al (2010) Nature Reviews Drug Discovery

MATERIALS and METHODS

Hurelflux Experiments

Table 1
Treatment of Human Co-cultures with TCA and TGZ for Biliary CL and Biliary Excretion Index (BEI) Determination

Treatment	TGZ Incubation (h)	TCA Uptake (min)
5 µM TCA (DMSO control)	ND	20
5 µM TCA + 10 µM TGZ	0.33, 2, and 72h	20

Table 2
Treatment of Human Co-cultures with TGZ and TGZ-SO_3 to Examine the Flux of Endogenous TCA[a] and d5-TCA[b]

Treatment	TGZ/TGZ-SO_3 Incubation (h)	TCA Uptake (min)
2 µM d5-TCA + 10 µM TGZ	0.17, 0.33, 2, and 24h	10
2 µM d5-TCA + 10 µM TGZ-SO_3	0.17, 0.33, and 2	10

[a]Endogenous TCA in hepatocytes
[b]5d-TCA added to medium compartment

Equations 1 and 2
Biliary Clearance and BEI Calculations

1. $\text{Biliary Clearance}_{\text{int}}\ (Cl_{int,biliary}) = \dfrac{\text{Accumulation}_{(Bile)}}{AUC_{media} \times f_u}$

2. $\text{Biliary Excretion Index (BEI)} = \dfrac{\text{Accumulation}_{(Bile)}}{\text{Accumulation}_{(Cells+Bile)}} \times 100$

* AUC_{media}: area under the substrate concentration-time curve (AUC)
* f_u: unbound fraction of substrate (f_u = 1 in the case of no BSA)

TGZ and TGZ-SO_3 Inhibition Experiments in Transfected HEK Cells

NTCP (Sodium-taurocholate cotransporting polypeptide) Inhibition Experiment
HEK cells transiently overexpressing NTCP (TransportoCells™) were purchased from Discovery Labware, Inc. The cells were thawed and plated on a 24-well Poly-D-Lysine plates. After approximately a 3-hour incubation at 37°C the seeding medium was changed with fresh medium supplemented with sodium butyrate. The cells were kept in a 37°C incubator with 8% CO_2 overnight. The inhibition experiment was initiated by adding HBSS buffer containing 5 µM TCA with 0 – 100 µM of TGZ and TGZ-SO_3. The incubation was terminated at 5 min by the removal of the assay buffer followed by washing the cells twice with ice-cold HBSS.

OATP1B1 Inhibition Experiment
HEK293 cells overexpressing OATP1B1 were seeded on poly-D-lysine coated 96-well plates at a density of 4.0 x 10^4 cells per well 48 hours before experiments. At the start of the experiment, cells were washed once with HBSS and incubated at 37°C with 5 µM TCA with 0 – 100 µM of TGZ and TGZ-SO_3. After 5 minutes, cells were washed twice with ice-cold D-PBS. The cells from the NTCP and OATP1B1 experiments were lysed and processed for LC-MS/MS analysis.

RESULTS

Figure 1
Biliary Clearance of TCA in Control Cells and Cells Treated with 10 µM TGZ

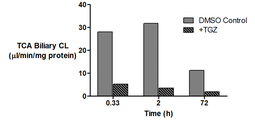
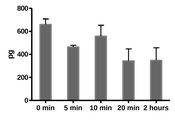


Figure 2
Amount of TGZ in Cells treated with 10 µM TGZ



RESULTS (continued)

Figure 3 a-d
Amount of Endogenous TCA and d5-TCA in Cells (a & c) and Bile (b & d) after Treatment with 10 µM TGZ and TGZ-SO_3

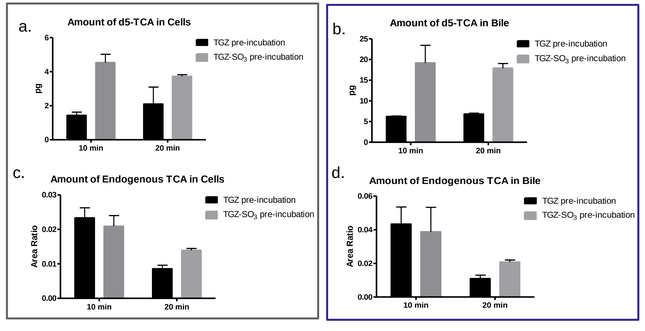


Figure 4 a-d
Amount of TGZ and TGZ-SO_3 in Cells (a & c) and Bile (b & d) after Treatment with 10 µM TGZ and TGZ-SO_3

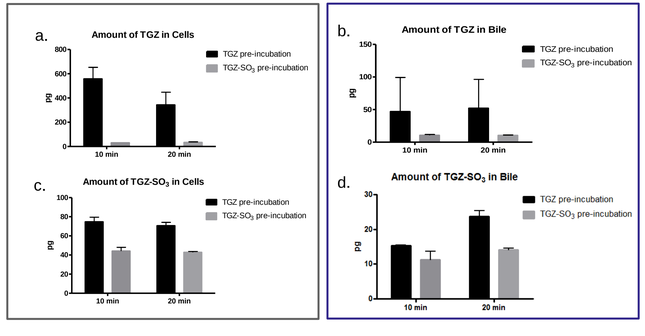


Figure 5
Inhibition of OATP1B1 Mediated Uptake of TCA in Transfected HEK Cells

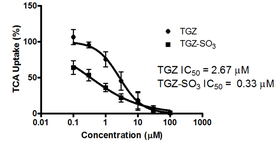
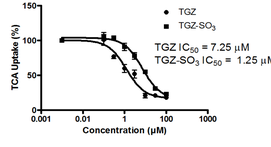


Figure 6
Inhibition of NTCP Mediated Uptake of TCA in Transfected HEK Cells



CONCLUSIONS

- Hurel human hepatocyte co-cultures readily metabolize troglitazone to its major circulating metabolite (TGZ-SO_3) and can form minor metabolites (TGZ-Q and TGZ-gluc).
- The BEI of TCA was comparable to the reported values in other models (SCH)
- TGZ and/or TGZ-SO_3 decreased the biliary clearance of TCA ≥5-fold between a 0.33 and 72-hour incubation.
- In OATP1B1 and NTCP transfected HEK cells TGZ-SO_3 is a more potent inhibitor of TCA uptake than TGZ.
- Endogenous TCA levels can be detected at low levels in cell and bile.
- It appears TGZ and its metabolites formed in cells are more potent than TGZ-SO3 alone at inhibiting 5d-TCA uptake into cells at 20 mins (Fig. 3a) thus reduced the 5d-TCA excreted into the bile (Fig. 3b).
- It also appears TGZ and its metabolites formed in cells are more potent than TGZ-SO3 alone at inhibiting the efflux of endogenous TCA into the bile (Fig. 3d) however a corresponding increase in cells is not observed (Fig 3c).

References

[1]Bi et al (2006) Drug Metabolism and Disposition
[2]Lee et al (2010) Journal of Pharmacology and Experimental Therapeutics
[3]Giacomini et al (2010) Nature Reviews Drug Discovery.
[4]Nozawa et al (2004) Drug Metabolism and Disposition

Acknowledgements

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