Mechanistic Modeling of Cyclosporine A-induced Acute Kidney Injury with RENAsym®

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ABSTRACT

OBJECTIVES: The use of Cyclosporine A (CsA) can cause tubular damage leading to a decline in renal function as determined by decreases in serum creatinine levels, glomerular filtration rate (GFR), and ATP[1]. This work uses RENAsym®, a quantitative systems toxicology (QST) model of acute kidney injury (AKI), to recapitulate clinical outcomes following CsA administration in humans.

METHODS: The effects of CsA on mitochondrial function and reactive oxygen species (ROS) production were assessed to define the potential for CsA-induced kidney injury. Human renal proximal tubule epithelial cells (RPTECs) were treated with CsA and its effects on mitochondrial respiration as well as ROS production were measured. Seahorse XF96 Analyzer was used to measure mitochondrial respiration. High content screening was used to measure ROS production after RPTECs were exposed to dihydroethidium staining. These *in vitro* data were used to define kidney toxicity parameters, and together with PBPK simulations of clinical CsA exposure created in GastroPlus®, kidney injury was predicted in RENAsym.

RESULTS: CsA inhibited the mitochondrial election transport chain flux (ETC inhibition coefficient=1458.33 µmol/L) and induced ROS production (V_{max}=0.049 1/hr, K_m =13.075 µmol/L). RENAsym predicted CsA-induced kidney injury such as a decrease in kidney average ATP as shown in Figure 4. RENAsym was further utilized to perform a mechanistic analysis to determine the main driver in simulated CsA nephrotoxicity. The mechanistic analysis indicated that CsA-induced kidney injury is primarily driven by inhibiting mitochondrial function via inhibition of the electron transport chain.

CONCLUSION: Using *in vitro* data to determine toxicity parameters, RENAsym accurately predicted CsA-induced nephrotoxicity in humans, consistent with observations from clinical studies

INTRODUCTION

Cyclosporine A (CsA) is an immunosuppressant known for inhibiting T-lymphocyte driven immune responses. CsA is commonly used following organ transplant to prevent organ rejection and in other diseases such rheumatoid arthritis, atopic dermatitis, and psoriasis. However, the use of CsA in humans, at doses range from 3 to 10 mg/kg, can cause nephrotoxicity. CsA can cause renal tubular damage subsequently leading to a decrease in renal function, indicated by an increase in serum creatinine levels and more importantly, a decrease in glomerular filtration rate (GFR)[2]. Here we use *in vitro* data and RENAsym®, a quantitative systems toxicology (QST) model of acute kidney injury (AKI), to recapitulate clinical outcomes following short-term CsA administration in humans.

DILI-sim Initiative

RESULTS

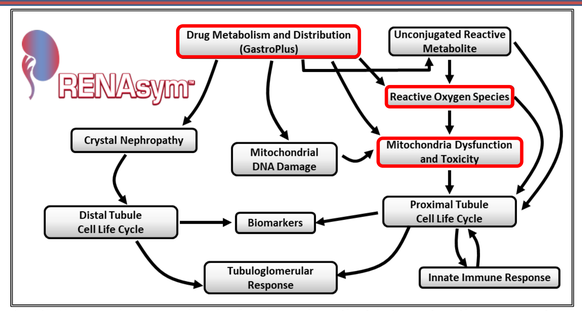

Figure 1: RENAsym is comprised of submodels that interact with one another to predict kidney injury outcomes. RENAsym combines data from *in vitro* toxicity studies, predictions of metabolism and distribution, as well as inner workings of kidney physiology to predict the potential for a given drug to induce acute kidney injury or cause nephrotoxicity.

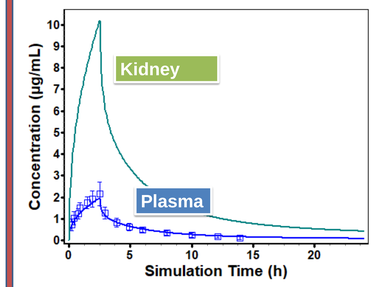

Figure 2: Simulated a single intravenous dose of CsA, 4 mg/kg, for 24 hours resulting in a plasma concentration in line with data[3] and predicted kidney concentration.

Using GastroPlus 9.7® we simulated a single IV dose of 4 mg/kg CsA for 24 hours. The ADMET Predictor module within GastroPlus predicted the kidney to plasma partition coefficient, Kp, to be 5.23. At a peak of 2.5 hours, the plasma is 1.96 µg/ml and in the kidney it is 10.2 µg/ml.

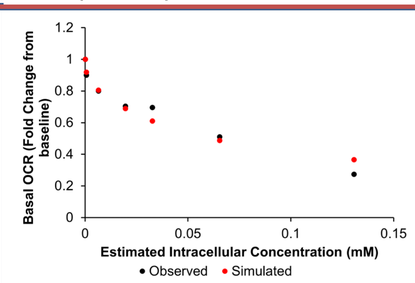

Figure 3: Simulated (red) and observed (black) basal oxygen consumption rate (OCR) (left) and reactive oxygen species (ROS) production (right) in RPTECs administered with 0.0125 to 25 µM of CsA.

CsA-induced electron transport chain (ETC) inhibition was simulated in MITOsym, and ROS production in RENAsym, against observed mitochondrial respiration and ROS production from *in vitro* studies. CsA inhibited the mitochondrial election transport chain flux (Non-saturable coefficient=1458.33 µmol/L, Saturable K_m=1.0488 µmol/L, Saturable V_{max}=0.3878 1/hr) and induced ROS production (V_{max}=0.049 1/hr, K_m =13.075 µmol/L)

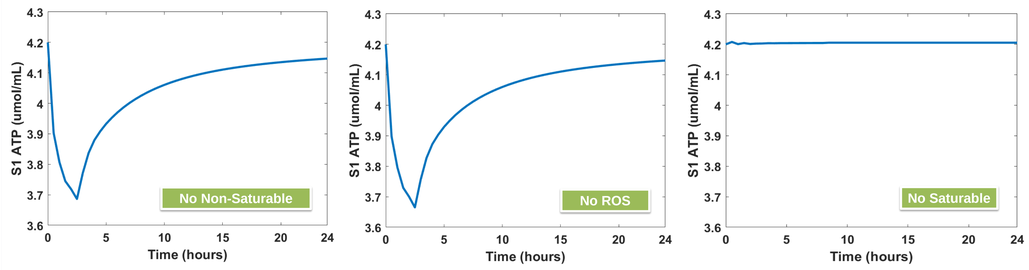

Figure 4: A mechanistic analysis was performed to predict which mechanism of toxicity was the primary driver in predicted CsA-induced nephrotoxicity. Simulations with no effect on non-saturable inhibition (left), no effect on ROS production (center), and no effect on saturable inhibition (right) were performed using RENAsym.

A single IV dose of CsA at 4 mg/kg was simulated in RENAsym with ROS and mitochondrial toxicity parameters. Each type of ETC inhibition, non-saturable or saturable, and ROS production was removed individually and then simulated in RENAsym. S1 ATP was used as a metric to compare contribution of each mechanism to simulated nephrotoxicity. When saturable inhibition was eliminated from simulations (right), the decrease in S1 ATP levels was predicted whereas, removing the other type of ETC inhibition and ROS production did not remove simulated nephrotoxicity. This suggests the effect of CsA on ETC is the primary driver in CsA-driven nephrotoxicity.

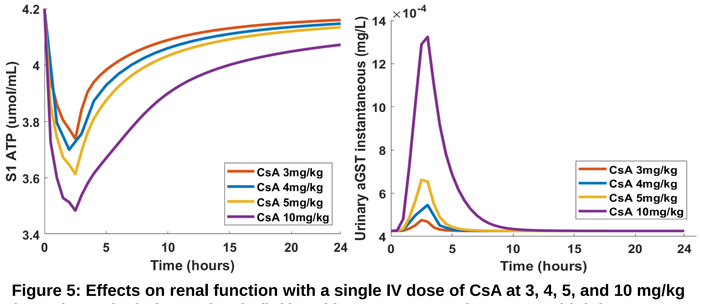

Figure 5: Effects on renal function with a single IV dose of CsA at 3, 4, 5, and 10 mg/kg for 24 hours include ATP levels (left) and instantaneous urinary αGST (right).

RENAsym was used to simulate a dose response of CsA exposure for 24 hours with a single IV dose of 3, 4, 5, or 10 mg/kg in humans. ATP levels decreased accordingly with an increase in dose. With a single dose of 10 mg/kg CsA, ATP levels dropped at 3 hours to 3.48 µmol/mL and did not return to baseline by 24 hours. An increase in urinary αGST, α-glutathione S-transferases, is a measure of tubular damage. Urinary αGST peaks at 3 hours for all four doses and levels return to baseline. Particularly, a single dose of 10 mg/kg peaks at 3 hours to 0.0013 mg/L and then returns to baseline.

METHODS

- Human renal proximal tubule epithelial cells (RPTECs) were treated with doses of CsA ranging from 0.01 to 25 µM.
- Mitochondrial respiration was measured using a Seahorse XFe96 Analyzer.
- Reactive oxygen species (ROS) production was measured using high content screening to quantify dihydroethidium staining following CsA exposure.
- PBPK simulations of a single dose of CsA was simulated in GastroPlus 9.7® for 3, 4, 5, 10 mg/kg.
- The kidney Kp for CsA was used to estimate intracellular concentration in toxicity assays, and toxicity parameterizations were based on intracellular kidney concentration.
- MITOsym® was used to parameterize ETC inhibition to *in vitro* mitochondrial respiration studies of CsA. ROS parameterization was performed in RENAsym.
- Simulations predicting kidney function and mechanistic analysis for CsA-induced nephrotoxicity were performed using RENAsym.

CONCLUSION

- Mechanistic analysis using RENAsym showed that inhibition of the mitochondrial electron transport chain is the primary mechanism responsible for the predicted decrease in kidney function.
- A dose response of CsA showed correlating decrease in renal ATP and an increase in urinary αGST.

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