Using Many In Vitro Assay Systems to Make a Kidney Toxicity Prediction of Doxorubicin: A Proof of Concept in Support of the New Approach Methodologies Initiative

Celeste Vallejo and Jeffrey L. Woodhead
Simulations Plus, Inc., 600 Park Offices Drive Suite 300 #4134 Durham, NC, 27713, USA
Contact: celeste.vallejo@simulations-plus.com

OBJECTIVE

- With the introduction of the new approach methodologies (NAMs) initiative, there is a focus on finding alternatives to animal studies to validate a drug's safety before administering in humans.
- Quantitative systems toxicology (QST) models are uniquely positioned to address this need. However, these models often rely on inputs from in vitro data with the ideal not always available.
- In this work, kidney toxicity predictions were made using information gathered in a variety of cell lines (kidney and others).
- **The objective of this proof of concept was to find ways to strengthen the predictive ability of QST models using less-than-ideal in vitro inputs.**

METHODS

- The kidney toxicity potential of doxorubicin (DOX) was examined in RENAsym v2A, a QST model of drug-induced kidney injury.
- DOX is a known kidney toxicant with a clinical observed toxicity rate of 23% after a therapeutic dose[1].
- Oxidative stress (ROS) and oxygen consumption rates (OCR) were measured in vitro in neonatal rat cardiomyocytes (NRCs)[2,3,4,5], H9c2 cells[6,7], and HK-2 cells[8,9] after incubation with DOX.
- Functions were fit to each of these data sets for use as input into the QST model.
- A PBPK model of DOX was developed to predict kidney concentrations, another input into the QST model.
- Kidney toxicity predictions after a clinically relevant dose of DOX were made using a simulated population (SimPops) with physiological variation to capture variability in a clinical population

RESULTS

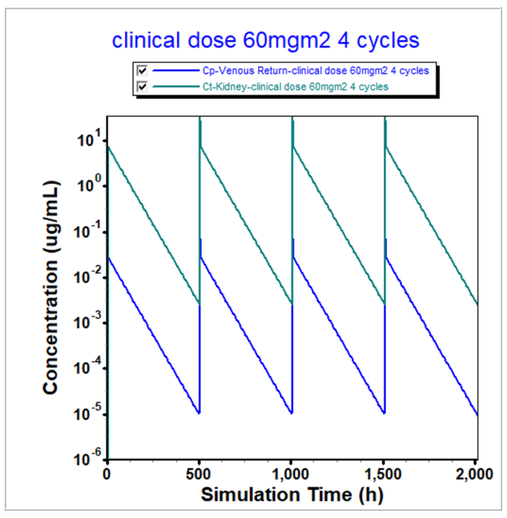


Figure 1. Plasma (blue) and kidney (green) concentrations after a simulated 60 mg/m^2 dose of DOX administered as an IV bolus on day 1 of each 21-day treatment cycle for 4 cycles using a PBPK model for a 70 kg patient with an assumed 1.79 m^2 BSA.

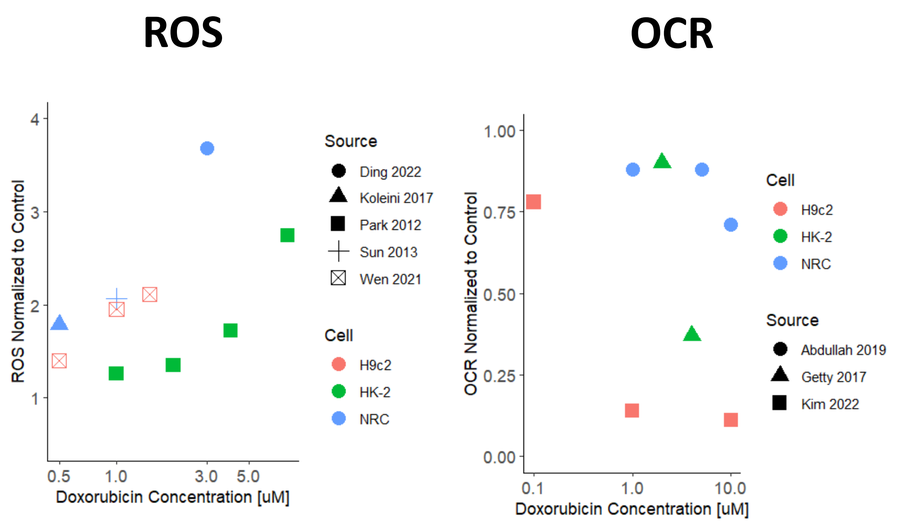


Figure 2. ROS and OCR in vitro data used to parameterize the toxicity pathways for each cell type. All cell types show DOX to increase ROS and inhibit the electron transport chain (ETCi).

Parameter	NRC	H9c2	HK-2 Scenario 1	HK-2 Scenario 2
Doxorubicin Molecular Weight [g/mol]	543.53	543.53	543.53	543.53
Kidney RNS/ROS production rate constant 1 [mL/nmol/hour]	0.0015	9.8E-4	8E-5	8E-5
Coefficient for ETC Inhibition 1 [mM]	NA	0.02	NA	NA
Coefficient for ETC Inhibition 3 [mM]	0.005	0.006	5E-4	5E-2
Max inhibitory effect for ETC Inhibition 3 [dimensionless]	0.65	0.999	0.63	0.68

Table 1. Toxicity parameterization based on the functions fit to the in vitro data in Figure 2.

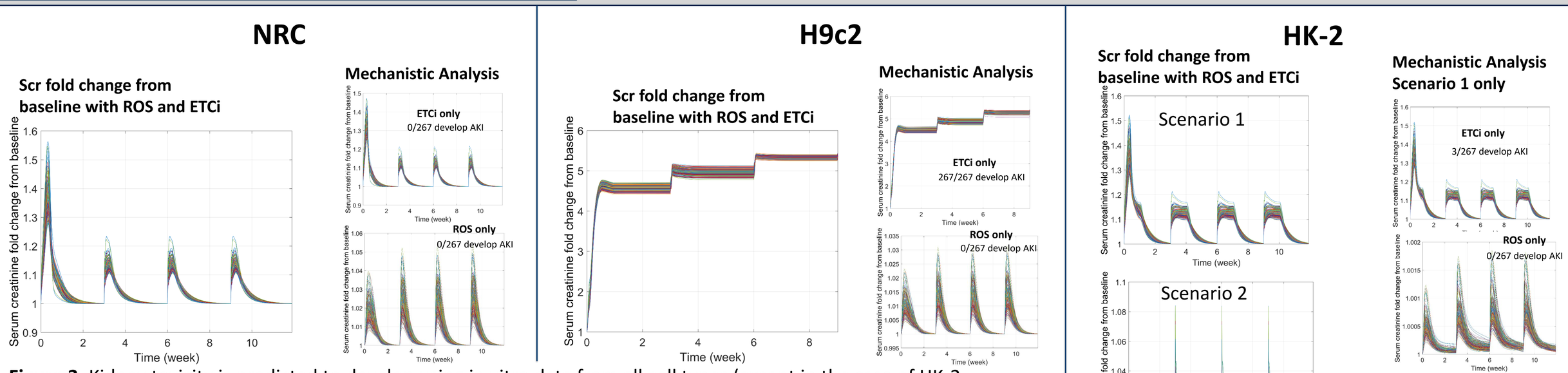


Figure 3. Kidney toxicity is predicted to develop using in vitro data from all cell types (except in the case of HK-2 Scenario 2) with incidence of 1.5%, 100%, and 1.1% in NRCs, H9c2, and HK-2 Scenario 1, respectively. Serum creatinine (Scr) fold change greater than 1.5x baseline within 48 hours of dosing[10] was used as the threshold for determining toxicity.

- After optimizing a PBPK model to DOX in vitro and in vivo data[11,12,13,14,15,16,17,18] along with data predicted from the molecular structure, the heart partioning coefficient (Kp) was predicted to be 106.6 and the kidney Kp was predicted to be 269.75
  - These values were used to estimate the kidney intracellular concentration used in the in vitro toxicity assays
- After a clinically-relevant dose (Figure 1), the predicted kidney C_{max} ranged from 10 to 34 µg/mL (18.4 to 63 µM)
- Functions were fit to the in vitro toxicity data (Figure 2) plotted against the estimated kidney intracellular concentration to parameterize each toxicity pathway in the QST model (Table 1)
  - Two scenarios for the OCR data in HK-2 cells due to the lack of data at lower concentrations were used to ascertain uncertainty
    - Scenario 1: OCR value at lowest measured concentration assumed to be constant over all lower concentrations
    - Scenario 2: OCR value declines 1 to value at lowest measured concentration across lower concentrations
- All three cell types predicted kidney toxicity due to DOX with incidence underpredicted in NRCs, overpredicted in H9c2s, and underpredicted in HK-2s compared to the clinical incidence (Figure 3)
  - A mechanistic analysis demonstrated ETCi to be the main driver of toxicity across all cell types

CONCLUSION

- All cell types indicated ROS and ETCi induced by DOX
  - The most ROS was induced in NRCs and the least in H9c2s
  - The most ETCi was induced in H9c2s and the least in NRCs
- In most cases, using in vitro data from any of the cell types would lead to a positive DOX-induced toxicity prediction but would seem as a rare event using NRCs or HK-2s and a guarantee using H9c2s
- **Take away:** in the case of DOX, a binary kidney toxicity prediction could be made using non-human and non-tissue specific cell lines

REFERENCES

1. Shavit et al. 2014. PMID: 24380911
2. Koleini et al. 2017. PMID: 29152091
3. Sun et al. 2014. PMID: 24502639
4. Ding et al. 2022. PMID: 35413642
5. Abdullah et al. 2019. PMID: 30765730
6. Wen et al. 2023. PMID: 36602546
7. Kim et al. 2022. PMID: 36277363
8. Park et al. 2012. PMID: 23028726
9. Getty 2017. https://mds.marshall.edu/etd/1137
10. Mehta et al. 2007. PMID: 17331245
11. Lee et al. 2010. PMID: 21175974
12. Seebacher et al. 2016. PMID: 27906178
13. Camaggi et al. 1988. PMID: 3162849
14. Mystridis et al. 2022. PMID: 35335919
15. Mross et al. 1990. PMID: 2076745
16. Mross et al. 1988. PMID: 3162516
17. Muller et al. 1993. PMID: 8339389
18. Gunven et al. 1986: PMID: 3719895

www.simulations-plus.com