Whether, How Much, and Why: Integrating Modeling and Systems Biology Approaches To Answer Questions About Use of Ursodiol in Neonates

Drug Development Advisors

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Objectives

- **Characterize the PK of ursodiol in neonates using PK modeling approaches.**
- **Demonstrate the usefulness of AMS as a tool for studying PK in neonates.**
- **Investigate dynamics of bile acid transport in a mechanistic physiological ("PhysioPD™") model.**
- **Incorporate metabolomic data for PhysioPD model calibration.**
- **Analyze possible causes of PK variability using PhysioPD model.**

Background

Pediatric Drug Development

Children are physiologically different from adults in ways that can affect drug metabolism and effects. Nonetheless, most medicines are currently prescribed to children in an off-label manner, with dosages extrapolated from adult data through body weight and surface-area calculations. This lack of PK information can result in adverse effects due to high doses, or suboptimal benefit due to inadequate doses.

PK assessment in neonates is difficult because:

- PK analysis requires frequent blood draws
- Standard assays require large blood samples
- Standard assay of radio-labeled drugs can result in significant exposure

Accelerator Mass Spectroscopy (AMS) is a technology that provides accurate PK measurement with much lower sample volume and exposure. A recent clinical trial sought to establish AMS as a tool for assessing drug PK in neonates.

Ursodiol and Cholestasis

Ursodiol (UDCA / Actigal®) is an endogenously produced bile acid approved to treat cholestasis (reduction of the normal flow of bile from the liver to the small intestine) in adults. It is frequently used off-label to treat neonatal cholestasis which is common in premature neonates admitted to the NICU. This is the first clinical study of UDCA PK in neonates.

Methods

- Use **mixed-effect** (NONMEM) **compartmental PK modeling** to estimate standard PK parameters.
- Use **mechanistic physiological ("PhysioPD™") modeling** to investigate the possible causes of PK variability and understand the dynamics governing bile acid transport in neonates.
- Incorporate data from bile acids **metabolomic analysis** into the PhysioPD model.

Clinical Trial

The Study was approved by the Loma Linda University Institutional Review Board and FDA-registered Radioactive Drug Research Committee. The study drug was synthesized in a radiochemistry laboratory (Moravek Biochemicals). Eligibility criteria included weight > 1,900g and no cholestasis. Neonate subjects were receiving parenteral nutrition (i.e., feeding tube). For more details, see 1.

Study Protocol:

- ^{14}C-ursodiol was administered to neonates via NG tube in three different doses (1, 3.3, or 10 nanoCuries of radioactivity; equal to 8, 26, or 80 nanograms of ursodiol) separated by intervals of 48 hrs.
- Blood samples (0.25 mL each) were collected at ≤ 0.5 hrs pre-dose and 0.5, 1.5, 3, 6, 12, and 24 hrs post-dose.
- Plasma was harvested and stored at -80º C until analysis.

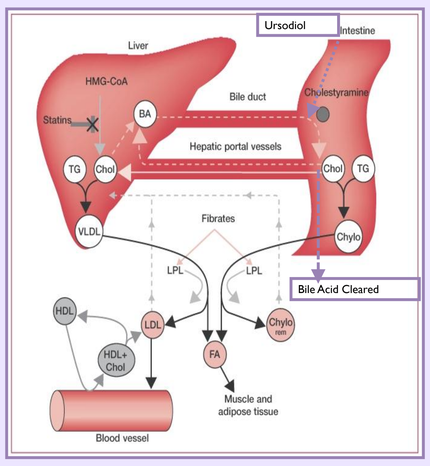


Figure 1. Graphical representation of bile trafficking. Ashley and Niebauer (2004) 5. Coronary artery disease. Cardiology Explained. London, Remedica. [cited 8/4/2010].

Trial Results

Table 1. Subject demographics

Subject Demographics	(n=5)
Gestational Age (weeks)	36 (35-40)
Weight at Study Entry (grams)	2,755 (1,910-3,180)
Gender (M/F)	3/2
Age at Study Entry (days)	2 (1-6)

Patient 4 was withdrawn from the study after the 2nd dose due to discharge, and Patient 5 was withdrawn after the 2nd dose due to withdrawn parental consent.

Ursodiol concentrations were detectable and highly variable across study subjects. While the doses administered were extremely small, the lowest measured drug concentration was significantly higher than the lower limits of quantification (LLOQ; Figure 2). This indicates that the total amount of labeled drug administered in future studies can be reduced, thus lowering the label exposure in newborns. Furthermore, smaller sample volumes may suffice in future clinical studies.

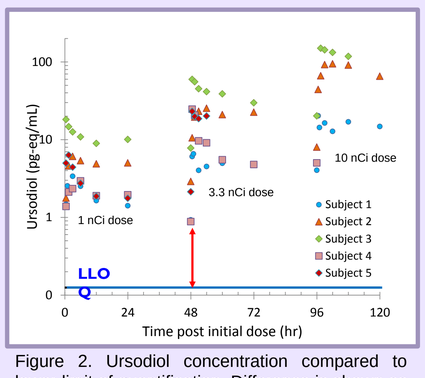


Figure 2. Ursodiol concentration compared to lower limit of quantification. Difference is shown as a red arrow.

PK Analysis

Mixed effect modeling identified a 2-compartment model as the best fit of the data, as can be seen in various diagnostic graphs. Furthermore, there was a generally good agreement with non-compartmental analysis (NCA, not shown, see 1) results of apparent CL and V values. Similar to the NCA results, large inter-individual variability in the PK parameters was identified.

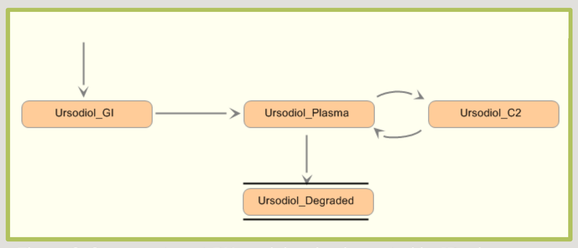


Figure 3. 2-compartment PK model as implemented in JDesigner software.

Table 2. Population PK parameters

Parameter	Estimate (%RSE)	IIV (%RSE)
ka (h^{-1})	1.65 (0.76)	-
CL (L/hr)	0.022 (0.39)	0.76 (0.68)
Vp (L)	0.935 (0.94)	1.2 (0.9)
Q (l/h)	1.24 (0.76)	-
V2 (L)	1.21 (0.19)	0.86 (0.5)

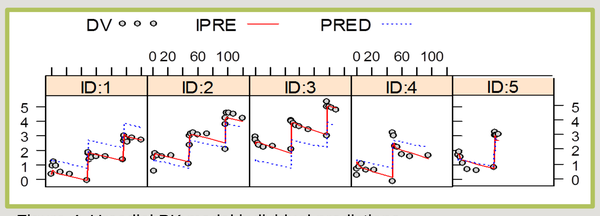
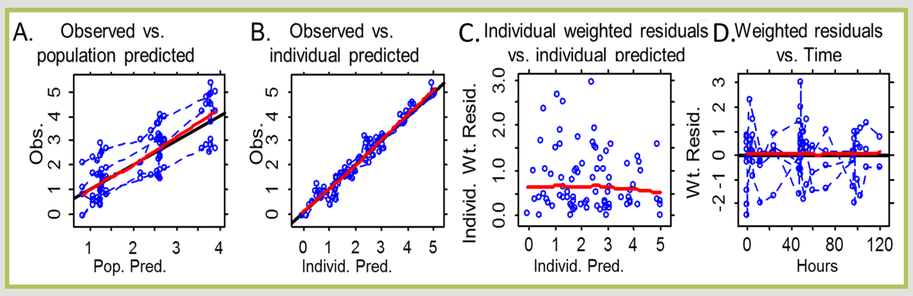


Figure 4. Ursodiol PK model individual predictions

Figure 5. Comparison of observed versus predicted values for the population model is variable as expected but clearly shows a trend (A). Comparison of the individual fit models with data shows a reasonable fit and trend (B). Comparison of the weighted residuals and either predicted values or time show no trends (C and D).



PK Conclusions

- **A 2-compartment model fitted the data best.**
- **AMS can be used to study complicated PK in neonates.**
- **The sample volume and dose of labeled ursodiol can be lowered for any future studies and still provide measurable data.**

PhysioPD Analysis

The PhysioPD modeling effort focused on developing a physiological model of bile acid transport which could be used to provide insights into:

- Bile acid transport in neonates
- Bile acid transport under parenteral feeding
- Observed variability in Ursodiol PK

The model was built and qualified for use in accordance with Rosa's Model qualification method (Figure 6). Research objectives, assumptions, design decisions, and data used are recorded in an MQM document that accompanies the model.

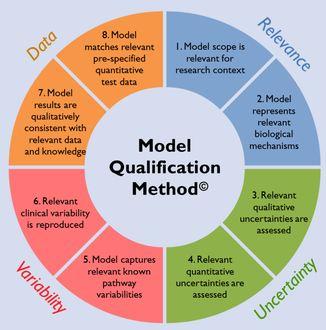


Figure 6. The Rosa & Co Model Qualification Method.

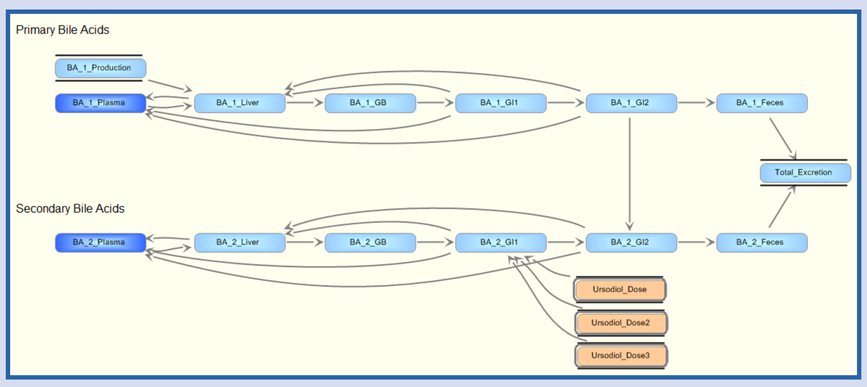


Figure 7. PhysioPD model of bile acid transport. Primary and secondary bile acids are represented. Bile acids transport goes from liver to gall bladder (GB) to GI tract. From the GI tract, bile acids can be transported back to liver or plasma or be excreted.

PK vs. PhysioPD Model Structure

The PK model and PhysioPD model both fit the data well (Figure 8), and both were implemented side-by-side in JDesigner to facilitate comparison. The PK model uses a standard two-compartment model structure, appropriate for deriving standard PK parameters. The PhysioPD model structure, in contrast, represents bile acid physiology and transport dynamics, which differ from the PK model in that:

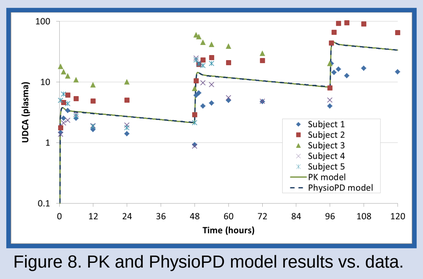


Figure 8. PK and PhysioPD model results vs. data.

- Dosing into the gut is represented as part of enterohepatic cycling mechanism
  - ➢ Initial appearance in plasma is physiologically linked to recirculation dynamics
- There is no clearance directly out of the plasma compartment
- The clearance mechanism is the final stage of the passage through the gut, but bile acids can also recirculate from the gut
  - ➢ Thus, clearance and recirculation rates are also physiologically linked

Sensitivity Analysis

Sensitivity analysis (SA) was performed to understand how variability in transport rates may explain observed variability in ursodiol. SA revealed that variability in different transport rates affect ursodiol dynamics in different ways. For example, varying the plasma to liver transport rate (Figure 9A) affects Cmax, tmax, distribution and clearance, while varying excretion rate (Figure 9B) affects only the terminal half-life.

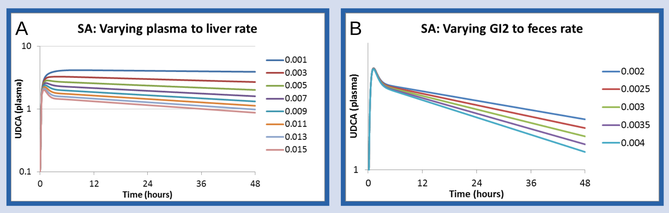


Figure 9. Effects on ursodiol concentration of varying plasma to liver transport (A) and excretion to feces (B).

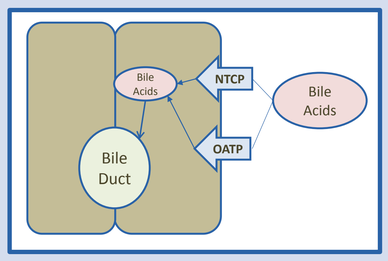


Figure 10. Physiological causes for variability in transport rate from plasma to liver. Given the sensitivity to plasma to liver transport, a likely explanation for the variability in the Cmax and distribution phase of ursodiol is polymorphisms in the organic anion transporter (OATP) and the sodium/bile acid cotransporter (NTCP)[8]. Pharmacogenomic data can thus be used to test model-based hypotheses.

Virtual Subjects

To explore what combinations of transport rates are consistent with the observed data, we created virtual subjects with variability in multiple transport rates. The resulting virtual subjects match observed dynamics (Figure 11) and can be used to understand the distribution of ursodiol across physiological compartments that is implied by the transport rates, which in turn may affect efficacy.

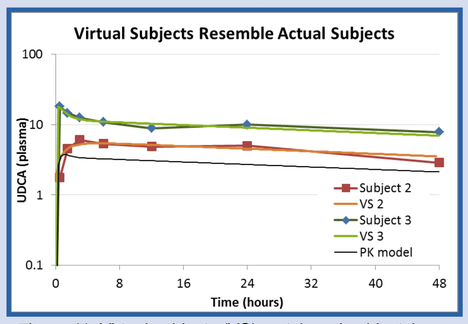


Figure 11. Virtual subjects (VS) match real subjects' ursodiol dynamics.

The virtual subjects' simulated results were compared to the ursodiol dynamics and to other data including synthesis, secretion, and recycling rates, and total pool sizes.[2-5,9-12] Metabolomic data[7] were particularly helpful because they provided a complete snapshot of all bile acid species in plasma.

Insights from PhysioPD Analysis

- Contrary to dogma regarding bile acid recycling and secretion under fasting:
  - Flux of bile acids into systemic circulation must be substantial to match drug appearance rates in plasma
  - Secretion rate out of gall bladder under parenteral feeding (i.e., no food in GI tract) must be substantial to match the recirculation rate (see Figure 12)
- Subject plasma profiles suggest variability in multiple transport steps
  - Suggests variability in distribution across compartments, which may have implications for drug efficacy
- Relative fluxes from GI to plasma vs. plasma to liver shape the initial peak; subjects with pronounced peaks have relatively fast plasma to liver transport, while subjects with no pronounced peak have relatively slow transfer from plasma to liver
  - Known polymorphisms may explain this variability (Figure 10)
- A fast terminal half-life suggests excretion rates greater than what is compatible with reported synthesis rates and equilibrium pool sizes
  - May point to differences between neonate and adult subjects, between fed and unfed subjects, ursodiol-specific regulation or other mechanisms

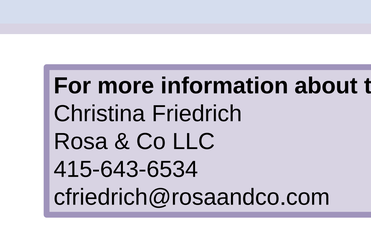


Figure 12. Gall bladder secretion rate was calibrated to data from fed subjects[10], shown as 1x. We expected the rate to be lower under parenteral feeding; however, model-based analysis shows that significantly lower rates (0.1x or 0.01x) are inconsistent with the dynamics and terminal half-lives observed. Further, such low secretion rates would imply sequestration of ~90% of the total bile acid pool in the gall bladder, which is inconsistent with other data. We conclude that gall bladder bile acid secretion during parenteral feeding is substantial.

- Next steps:
  - Incorporate efficacy data (currently being collected)
  - Investigate how transport rate variability may affect drug concentration at sites of action and hence efficacy
  - Optimize dosing and protocol (e.g., fed vs. unfed)

PhysioPD Conclusions

- **A mechanistic physiological (PhysioPD) model of bile acid metabolism can match data as well as a standard PK model and give physiological insights**
- **Recycling via enterohepatic circulation is substantial even during fasting**
- **Virtual subjects that match real subjects can be used to explore the underlying causes of observed variability**
  - **Pharmacogenomic data suggest that polymorphisms in the OATP and NTCP transporters are probable causes accounting for some ursodiol PK variability**
- **Metabolomic data can easily be incorporated into PhysioPD models**
- **The combination of PK modeling and PhysioPD modeling provides a standard set of parameters for characterizing PK and a means to investigate underlying causes of variability**

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