

Objectives

- Investigate the Pharmacokinetics (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.
- Analyze possible causes of PK variability using PhysioPD model.

Background

Pediatric Drug Development

Children are physiologically different from adults; therefore, results from drug studies conducted in adult populations cannot simply be extrapolated to pediatric populations. Many new drug applications still do not adequately address pediatric assessment, and little or no pediatric data exists for drugs approved before the Pediatric Research Equity Act (PREA). As a result, most medicines are prescribed to children in an off-label manner, with dosages extrapolated from adult data through body weight and surface-area calculations. The lack of accurate PK information can result in adverse effects due to high doses, or suboptimal benefit due to inadequate doses. One of the reasons for a lack of clinical drug testing in newborns is the volume of blood necessary for PK studies, typically 100 to 500 µl per study in adults, presents a major obstacle to the study of PK in neonates.

Cholestasis

Cholestasis (reduction of the normal flow of bile from the liver to the small intestine) is a common affliction of premature neonates admitted to the neonatal intensive care unit. Ursodiol (Actigal®) is an endogenously produced bile acid approved to treat cholestasis in adults is frequently used off-label to treat neonatal cholestasis.

The mechanism of action of ursodiol for treatment of cholestasis in neonates is unknown. Its efficacy varies widely from patient to patient. The PK of UDCA in neonates, which may explain its wide variation in efficacy, have never been characterized because measurement requires differentiating between endogenous and exogenous compound by using a labeled tracer.

This is the first clinical study of UDCA PK in neonates.

Ursodiol (ursodeoxycholic acid) is a normal part of human bile. Ursodiol is used to treat:

- Gallstones
- Primary biliary cirrhosis
- Biliary atresia
- Parenteral nutrition-associated cholestasis

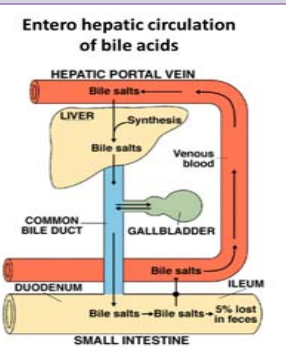


Figure 1. Graphical representation of bile trafficking. Vander/ Sherman/ Luciano. Human Physiology, 7th edition. McGraw Hill, 1998.

In adults, Ursodiol functions by:

- Inhibiting cholesterol absorption
- Suppressing cholesterol synthesis, secretion
- Solubilizing cholesterol
- Reducing bile viscosity
- Increasing bile flow

Clinical Trial

This 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 (Moravex Biochemicals).

Eligibility Criteria:

- Neonates admitted to the LLUCH Neonatal ICU
- No cholestasis
- Weight \geq 1,900 g
- Vascular indwelling catheter for blood draws
- Nasogastric tube in place
- Hemoglobin $>$ 11 g/dL

Exclusion Criteria:

- Major gastrointestinal congenital anomalies
- Neonatal hepatitis
- Anatomic evidence of bile duct obstruction (biliary atresia)
- Cholelith cyst
- Symptoms suggestive of cystic fibrosis

Study Protocol:

- 14 C-ursodiol was administered to neonates via NG tube in three different doses (1, 3.3, or 10 nanoCuries of radioactivity, 8, 26, or 80 nanograms of ursodiol) separated by intervals of 48 hrs.
- Blood samples (0.25 ml each) were drawn \leq 0.5 hrs before each dose administration, then again at 0.5, 1.5, 3, 6, 12, and 24 hrs after each dose.
- Blood samples were centrifuged to separate serum from RBCs and then stored separately at -80° C until AMS analysis.
- 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.

References used to build and test the model:

- Cornes, R. L., Cantliffe, J., et al. (1979). "Biliary bile acids in primary biliary cirrhosis: effect of ursodeoxycholic acid." *Hepatology* 29(6): 1549-1554.
- Emerson, K. S., S. M. Gandy, et al. (1979). "Enterohepatic circulation rates of cholic acid and chenodeoxycholic acid in man." *Gut* 20(12): 1078-1082.
- Frachiza, M. K., D. Sechel, et al. (1996). "Bile acid conjugation in early stage cholestatic liver disease before and during treatment with ursodeoxycholic acid." *Clin Chim Acta* 248(2): 175-185.
- Hamilton, J. P., G. Xu, et al. (2007). "Human fecal bile acids: concentration and spectrum." *Am J Physiol Gastrointest Liver Physiol* 293(1): G259-263.
- Northcutt, T. C., and A. F. Hofmann (1978). "Biliary lipid output during three meals and an overnight fast. I. Relationship to bile acid pool size and cholesterol saturation of bile in gallstone and control subjects." *Gut* 19(1): 1-11.
- Rosa, E., R. Andri, et al. (1970). "Enterohepatic circulation of bile acids after cholecystectomy." *Gut* 11(7): 640-649.
- Thistle, J. L., N. F. Laursen, et al. (1982). "Differing effects of ursodeoxycholic or chenodeoxycholic acid on biliary cholesterol saturation and bile acid metabolism in man: A dose-response study." *Gut* 33(2): 161-168.
- Tonei, D., E. Gallazzi, et al. (1997). "Bile acid kinetics in man studied by radio thin-layer chromatography and densitometry coupling." *J Chromatogr B Biomed Sci Appl* 700(1-2): 93-94.

Modeling Analysis

- Non-compartmental analysis was conducted using PK solver.
- Compartmental analysis was conducted using Nonmem.
- PhysioPD models were built using JDesigner part of the Systems Biology Workbench, using data from literature [1,2].

Results

Demographics

A summary of the demographics of enrolled subjects is shown in Table 1. 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.

Table 1. Subject demographics

Patient Demographics (n=5)	
Gestational Age (weeks)	36 (35-40)
Weight at Study Entry (grams)	2,755 (1,910-3,180)
Height (cm)	45 (42-47)
Body Surface Area (m ²)	0.189 (0.151-0.208)
Haycock et al. Gender (n = M/F)	5 (3/2)
Age at Study Entry (days)	2 (1-6)

Preliminary Data Analysis

Initial evaluation of the data showed that while the doses administered were extremely small, the lowest measured drug concentration was significantly higher than the lower limits of quantification (LLOQ). 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 be needed in future clinical studies.

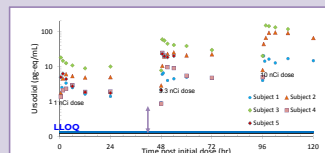


Figure 2. Ursodiol concentration compared to lower limit of quantification. Difference is shown as a red arrow.

Non-compartmental Analysis

Non-compartmental analysis (NCA) was conducted on the drug concentration data for the 8 ng dose. Each subject was evaluated separately and the results for all five subjects included in the analysis. A large variability was observed in the estimated parameters.

Table 2. NCA results

Parameter	Unit	Median	Mean	SD
t1/2	h	42.95	44.13	14.15
Imax	h	3.00	2.70	2.17
Cmax	ng/L	6.08	7.37	6.21
lag	h	0	0	0
AUC 0-inf	ngmL/h	226.45	417.72	452.79
CL/F	L/hr	1.56	2.05	1.19
CL/F	L/hr	0.035	0.038	0.026

Compartmental 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.

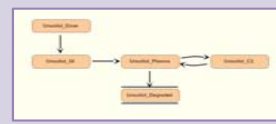


Figure 3. 2-compartment PK model in JDesigner software.

Table 3. Population PK parameters

Parameter	Estimate (%RSE)	IV (%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 (mL/h)	1.24 (0.76)	-
V2 (L)	1.21 (0.19)	0.86 (0.5)

Figure 4. 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).

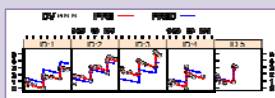


Figure 5. Ursodiol PK model individual predictions

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 metabolism which could be used to provide actionable insights into

- The variability in Ursodiol PK
- The biological effects of Ursodiol on bile acid metabolism (Ursodiol MOA)
- Incorporation of metabolomic data into a physiological model
- Translation of metabolism and PK between adult and pediatric models

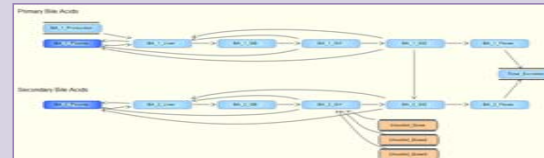


Figure 6. Graphical view of the PhysioPD model of bile acid transport with primary and secondary bile acids represented. Bile acids transport goes from liver to gall bladder (GB) to GI tract. From the GI tract, bile acids can be transported back to liver or plasma or excreted.

The PK model and PhysioPD model both fit the data well (Figure 7), 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

- Dosing in the gut is represented as part of enterohepatic recycle, linked to recirculation.
- There is no central (plasma) clearance.
- Clearance is part of the last stage of passage through the gut, linking clearance to recirculation.

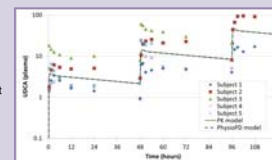
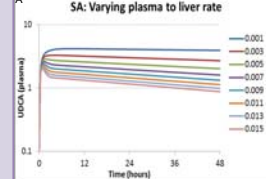


Figure 7. PK and PhysioPD model results vs. data.

SA: Varying plasma to liver rate



SA: Varying GI2 to feces rate

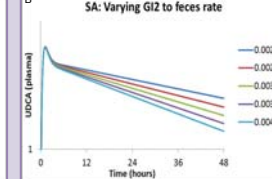


Figure 8. Effects on ursodiol concentration of varying plasma to liver transport (A) and excretion to feces (B).

Sensitivity analysis (SA) was performed to understand how variability in transport rates may explain observed variability in ursodiol time-concentrations. Analysis revealed that variability in different transport rates affect ursodiol dynamics in different ways. For example, varying the plasma to liver transport rate (Figure 8A) affects Cmax, tmax, distribution and clearance, while varying excretion rate (Figure 8B) affects only the terminal half-life.

Virtual Subjects Resemble Actual Subjects

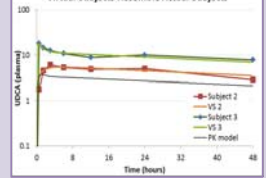


Figure 9. Virtual subjects (VS) match real subjects' ursodiol dynamics.

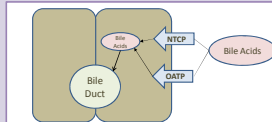


Figure 10. Physiological causes for variability in transport rate from plasma to liver. Given the sensitivities of the model variability in the Cmax and distribution phase of ursodiol is likely due to polymorphisms in the organic anion transporter (OATP) and the sodium/bile acid cotransporter (NTPC). Pharmacogenomic data was used to test this model-based hypothesis.

The virtual subjects' simulated results were compared to the ursodiol dynamics and to other data including synthesis, secretion, and recycling rates, and total pool sizes²⁴. Metabolomic data were particularly helpful because they provided a complete snapshot of all bile acid species in plasma (data not shown).

PhysioPD Conclusions

- Both standard PK and PhysioPD models represent ursodiol PK data well
- In addition, the PhysioPD model provided physiological insights:
 - Enterohepatic recycling is substantial, even during fasting
 - Virtual subjects matching real subjects were used to explore underlying causes of observed variability
 - Ursodiol PK variability is likely due to OATP and NTPC transporters, consistent with genomic data
- 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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