An In Vitro Model Simulating Gastro-Intestinal Digestion in Neonates and Young Infants (2 Months Old or Younger) and Findings with Study Test Model Drugs: Furosemide, Fluconazole and Ibuprofen

Kofoed-Djursner, Caroline[1]; Berthelsen, Ragna[1]; Selen, Arzu[2]; Müllertz, Anette[3]. 1:Department of Pharmacy, University of Copenhagen, Denmark, 2: FDA/CDER, Silver Spring, Maryland, USA, 3:Bioneer: FARMA, Department of Pharmacy, University of Copenhagen, Denmark

Abstract

Plain Language Synopsis:
Development of dosage forms for pediatric patients benefit from creative, reliable and informative methodology for achieving the intended therapeutic benefit. An in vitro gastrointestinal model was developed and applied for studying factors that may influence drug solubilization under conditions that may be encountered in vivo in neonates and young infants.

Background: The coining of "therapeutic or pharmaceutical orphans" by Harry Shirkey initiated dedicated efforts, regulations and research to advance drug development for pediatric patients. Due to the inherent complexities of conducting studies in pediatric patients, an in vitro modeling approach is described here for providing knowledge to support design of informed and targeted in vivo study(ies) for pediatric patients.
Purpose: To assess the performance of the developed in vitro gastrointestinal (GI) digestion model and explore factors that may influence in vitro and in vivo solubilization (i.e., bio-accessibility) of drug substances in neonates and young infants.
Methodology: The dynamic in vitro lipolysis model (Klitgaard et al. 2017) was used for testing study model drugs (furosemide, ibuprofen and fluconazole). Their solubilization and phase distribution were studied in various media (e.g., infant formula, and fed and fasted state simulated gastric and intestinal biorelevant media with and without digestive enzymes) with varying viscosity.
Results: Furosemide: Displayed higher solubilization in the aqueous phase in fed state than "fasted state" (i.e., pre-feeding). Furosemide solubilization as drug substance or crushed tablet increased in the presence of food (infant formula) but was not further influenced by the GI digestion of the infant formula.
Ibuprofen: All drug was solubilized in the aqueous phase of the gastric and intestinal steps in fed state experiments. However, in "fasted state" experiments, in the gastric step, ibuprofen precipitated completely and redissolved in the intestinal step where 40% of ibuprofen was solubilized in the aqueous phase. Digestion products decreased the amount of ibuprofen in the aqueous phase, as ibuprofen partitioned between the lipid and the aqueous phase.
Fluconazole: Fully solubilized in the aqueous phase in both gastric and intestinal steps and was unaffected by the altered test conditions.
Conclusions: The developed GI digestion model may inform in vitro drug product performance by mimicking feeding patterns and processes occurring in the GI tract of the neonates and young infants. Integration of such knowledge with intended therapeutic outcomes can advance building in clinical relevance in development of dosage forms for neonates and young infants with greater therapeutic benefit, and ultimately, facilitate better informed regulatory decision-making.

Introduction

Harry Shirkey's focus in 1968 (1) on the lack of drug products labeled for pediatric patients led to many collaborations, regulations and dedicated efforts and research for increasing the number of dosing recommendations and drug products labeled for pediatric patients. Collective efforts and commitment of the pediatrics community and the stake-holders led to the first two laws Best Pharmaceuticals for Children Act (BPCA) and the Pediatric Research Equity Act (PREA). Efforts are continuing with additional focus on advancing dosing recommendation and drug labels for neonates (2).

Why this project?

A possible path for developing dosing recommendations for the young and the youngest pediatric patients.

1) Conducting studies in pediatric patients (particularly the youngest), even non-noninvasive, are difficult and require specific set-up, skills and clear critical questions for which the answers can be verified.
2) The in vitro methods simulating in vivo gastro-intestinal environment in neonates and young infants (0-2 months old) under various feeding conditions (fed or fasted (i.e., just before feeding), and impact of factors on drug solubilization can inform us on
   a) the interface between the patient characteristics and the drug substance and drug product performance in an environment consistent with those observed in neonates and young infants (0-2 months old) and
   b) combined or varied effect of factors such as type and frequency of meals and feeding patterns on dissolution, solubilization and the extent of available drug for absorption (i.e., bioaccessibility) and
   c) the likely optimal conditions for oral dosing of the intended therapeutic dose and the dosage form

Studying and verifying the optimized dose and dosage forms can result in more drugs with labeling information for their use in this youngest patient population.

Materials and Methods

Overview:

- The model developed here is based on the dynamic in vitro lipolysis model (3). The information needed for the in vitro gastrointestinal (GI) model was extracted from literature. The average body weight of 2 kg for a neonate is determined from the body weight of neonates (n=482) obtained from multiple publications (4). The two approaches for the developed model are shown in Figure 1.
- Drug substances (furosemide, fluconazole and ibuprofen), drug products (furosemide and ibuprofen tablets and fluconazole powder for oral suspension), baby/infant formula (Néstle NAN-1®), the experimental supplies such as Nestle's ThickenUp® containing Xanthan gum and the laboratory supplies were purchased and used in the studies conducted by the University of Copenhagen.
- Sample preparation and analytical details are included in the references (4-6). The quantification of ibuprofen and fluconazole was performed by UPLC and by HPLC for furosemide with UV-detection. The range of analytical standards used for method validation and quantitation of drug concentrations in the samples bracketed the concentration range of the study samples.
- For bringing in a pediatric context in the digestion experiments, "dose equivalents" were estimated for each drug (i.e., a likely pediatric dose for a neonate; 3 mg/kg for furosemide, 3 mg/kg for fluconazole and 10 mg/kg for ibuprofen). The recoveries in the digestion experiments are reported as a percentage of the estimated dose equivalent for a 2 kg neonate.
- To ensure adequate sampling volume in the experiments, the dose equivalents and the fluid volumes are scaled up six times compared to the values provided in Table 1.
- The media simulating gastric and intestinal steps under fed and fasted conditions are listed in Table 1. The properties of the study test model drugs are presented in Table 2. The generated in vitro results are presented in Figures 2 and 3.

Table 1

	Pediatric Fed State Media Final*		Pediatric Fasted State Media Final*	
Components	Gastric Step	Intestinal Step	Gastric Step	Intestinal Step
NaCl (mM)	3	89.5	160.3	90.2
TRIS (mM)	2	2	-	2
Maleic Acid (mM)	2	2	-	2
Sodium Taurocholate (mM)	-	1	-	4.7
Phospholipids (mM)	-	0.2	-	1.0
Enzymes				
Gastric lipase (TBU/mL)	17	-	100	-
Pepsin (U/mL)	126	-	741.2	-
Pancreatic lipase (TBU/mL)	-	50	-	250
Volumes				
Food (Milk/infant formula) (mL)	47	-	-	-
Medium (mL)	2.9	22.2	2.9	9.4
Other physiological factors				
pH	6.4	6.5	2.8	7.0
Osmolality (mosmol/kg)	291	296	298	308

The Two Digestion Models: Immediate and Continuous Transfer (ITM and CTM)

(The results from ITM are presented)

1) Briefly, furosemide and ibuprofen drug substances as powder or crushed tablets (data not shown), and fluconazole reconstituted powder for suspension (10 mg/mL)) were added as dose equivalents either to 1.5 mL water (for fasted studies) or into baby formula (23.5 mL/kg body weight of neonate) for fed studies.
2) Depending on fed or fasted conditions and gastric and intestinal steps, enzymes as listed in Table 1 are introduced into the same temperature- and pH- controlled reaction vessel, for gastric and intestinal steps in sequence for the immediate transfer model (ITM). Gastric digestion step continues for 50 min. The intestinal digestion step starts with addition of the concentrated intestinal media containing the intestinal digestion enzymes and adjusting of the pH of the medium by automated addition of NaOH (0.1 M) for fasted state experiments. The intestinal step continues from t=51 to t=111min. Similarly, fed state experiments are carried out with digestion medium described in Table 1.

Table 2

Properties	Furosemide	Fluconazole	Ibuprofen
Mw (g/mol)	330.7[1]	306.3[2]	206.3[3]
pKa (25°C)	3.60, 10.15[1]	1.76[2]	4.5[3]
Nature	Acid[1]	Basic[4]	Acid[1]
logP	2.56[1]	0.5[2]	4.0[3]
Solubility in water (µg/mL)	18.3[5]	6 mg/mL	21.0[5]
Solubility in FaSSGF (µg/mL)	13 ± 2	> 1 mg/mL	33 ± 1
Solubility in FeSSGF (contains high fat milk (i.e., 3.5%)) pH 5 (µg/mL)	412 ± 13	> 1 mg/mL	1601 ± 85
Solubility in modified FeSSGF (infant formula, 3.5%) pH 5 (µg/mL)	721 ± 19	> 1 mg/mL	3127 ± 239
Solubility in modified FeSSGF (3.5%) pH 6.4 (µg/mL)	4487 ± 207	> 6 mg/mL	>6 mg/mL

Results and Discussion

The results presented in this poster were obtained by application of the Immediate Transfer Model (Reference 4).

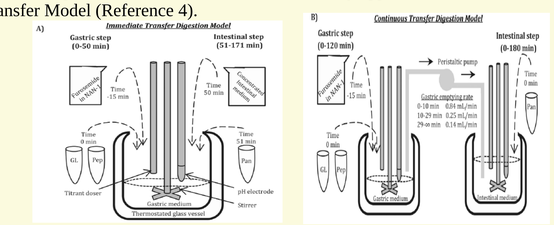


Figure 1: The Immediate Transfer Model (ITM) (A) and the Continuous Transfer Model (CTM) (B)

Comparing/contrasting solubilization and the effect of digestion under fed (with and without digestive enzymes) and fasted (i.e., pre-feeding) conditions with Immediate Transfer Model

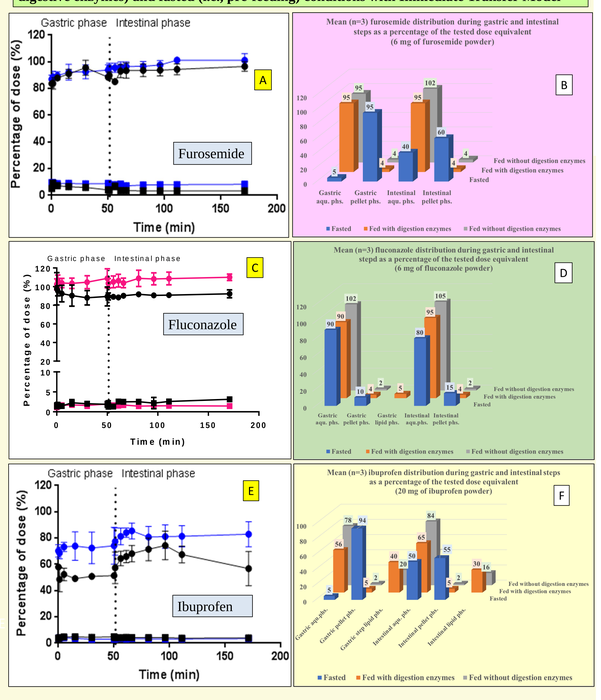
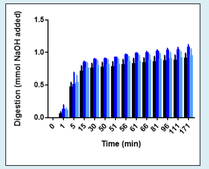


Figure 2: Digestion experiments illustrating solubilization of the study test model drugs under fed conditions with and without digestive enzymes for furosemide (A), fluconazole (C) and ibuprofen (E) and recoveries in aqueous, pellet and lipid phases (as applicable) for furosemide (B), fluconazole (D) and ibuprofen (F) under fasted (i.e., before feeding) and fed conditions with and without digestion enzymes using ITM. Distribution of the study test model drugs are shown with solid circles into the aqueous phase, solid squares into the pellet phase and with blue or red solid circles into the aqueous phase in fed state experiments conducted without the digestion enzymes. The mean(n=3) ± SD values are illustrated at the sampling times.



The extent of digestion is determined based on the added NaOH (0.5 M) for maintaining constant pH during the gastric and intestinal phases of the fed state experiments. The automated addition of NaOH corrects the drop in pH due to released free fatty acids into the medium. The extent of digestion observed for one of the study test model drugs is shown as an example in Figure 3.

Figure 3: The mean (n=3) (SD) NaOH added to maintain constant pH during the digestion of baby/infant formula (NAN-1) for fluconazole powder (black bars), fluconazole STADA® capsule contents (dark blue bars) and fluconazole suspension (10 mg/mL) (light blue bars).

Drug Powder	Mean (n=3) (± SD) Polymer content w/v Néstle ThickenUp®			
	Control	0.1%	0.25%	0.5%
Furosemide	89% ± 15%	77% ± 25%	57% ± 22%	94% ± 26%
Fluconazole	101% ± 29 %	137% ± 29%	79% ± 51%	61% ± 17%
Ibuprofen	100% ± 22 %	62% ± 11%	48% ± 11%	77% ± 15%

Table 3: The results are from the viscosity experiments exploring the effect of increasing viscosity on recovered dose equivalents (as percentage of the dose equivalents) of the study test model drugs. The range of viscosity of the digestion medium varied from ready-to-drink infant formula to drinkable yogurt.

Conclusions

1) Assessment of the *in vitro* GI solubilization and digestion model with the study test model drugs (furosemide, fluconazole and ibuprofen) shows that the results are reproducible, and that the system is versatile and sensitive to drug substance properties and perturbations (e.g., increasing viscosity of the digestion medium). The developed ITM and CTM approaches can facilitate studying the impact of factors such as gastric emptying, feeding patterns, characteristics and composition of the meals, characteristics of drug substance and drug product, unique attributes of the targeted patient population, and ultimately, provide information on the patient and drug product interface.
2) Under fed conditions, furosemide aqueous solubility increased significantly compared to fasted conditions and is not affected significantly by the digestion products; whereas for ibuprofen, its aqueous solubilization also increased under fed conditions, however, due to digestion of the infant formula and partitioning of ibuprofen into the lipid phase, recovery of ibuprofen in the aqueous phase was reduced relative to "fed" conditions without digestive enzymes. In the case of fluconazole, it is highly solubilized in the aqueous phase under fasted and fed conditions (with and without digestive enzymes).
   Changes in viscosity perturbed the system, affected solubilization of the three study test model drugs as expected, and affected the recoveries in the aqueous phase and increased the variability in the solubilization data while providing a comparative landscape for the observations.
3) These results warrant continuation of efforts to advance the developed methodology, which can be utilized for generating specific data and addressing critical questions related to factors influencing drug absorption in targeted patient populations.

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DISCLAIMER:

The approaches and conclusions in this presentation have not been formally disseminated by the United States Food and Drug Administration and should not be construed to represent any Agency determination or policy.

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