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Studying furosemide solubilization using an *in vitro* model simulating gastrointestinal digestion and drug solubilization in neonates and young infants

Mette Klitgaard[a], Philip Jonas Sassene[a], Arzu Selen[b], Anette Müllertz[c], Ragna Berthelsen[a,*]

[a] Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark
[b] Office of Testing and Research, US Food and Drug Administration, Center for Drug Evaluation and Research, Silver Spring, MD, United States
[c] Bioneer:FARMA, Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark

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ABSTRACT

Objective: The aim of the present study was to study the oral performance of furosemide in neonates and young infants using a newly developed *in vitro* model simulating digestion and drug solubilization in the gastrointestinal (GI) tract of the human neonate and young infant population (age 0–2 months).

Methods: The utilized *in vitro* model was designed to mimic the digestion and drug solubilization processes occurring in the stomach, and the small intestine of the neonate and young infant population, using physiologically relevant media, volumes and digestive enzymes. Overall the experimental model setup was based on the dynamic *in vitro* lipolysis model previously described by Fernandez et al. (2009). The amount of furosemide solubilized in the aqueous phase during a digestion study was used as an estimate for the amount of drug available for absorption *in vivo*. By varying different factors in the model setup, *e.g.* presence of food (food-effect), effect of digestion (tested with and without addition of digestive enzymes), and properties of the dosage form, it was possible to estimate the importance of these factors *in vivo*.

Key findings and conclusions: The present *in vitro* data suggest that the oral performance of furosemide in neonates and young infants will be increased by the presence of food (frequent feedings) due to increased drug solubilization, however, not influenced by the GI digestion of this food. The properties of the dosage form (immediate release tablets) did not affect the drug solubilization as compared to administration of the pure drug powder.

1. Introduction

Within the more recent years, there has been an increasing focus on drug delivery to the pediatric population. In order to ensure high-quality information and research on medicines administered to the pediatric population, specific guidelines and requirements on children's medicine have been set out by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (Zisowsky et al., 2010; European Medicines Agency, 2006; European Medicines Agency, 2016; European Medicines Agency, 2007; U.S.Department of Health and Human Services Food and Drug Administration, 2005; Administration USDoHaHSFaD, 2014; U.S.Department of Health and Human Services Food and Drug Administration, 2015). As part of these requirements, the development plan of any new medical product must include an initial pediatric study plan including timing of studies in clinical, non-clinical and technical aspects, as well as measures planned to demonstrate quality, safety and efficacy for the pediatric population (Zisowsky et al., 2010; The European Commision. Better Medicines for Children From Concept to Reality, 2013; Administration USDoHaHSFaD, 2016).

In vitro models simulating drug behavior in the gastrointestinal (GI) tract are commonly used tools for estimating oral drug performance prior to conducting *in vivo* studies. These models become increasingly important for the pediatric population, as very strict ethical concerns are connected with clinical studies in this particular population. As drug absorption is typically rate limited by the drug dissolving in the GI fluids (dissolution or solubility rate limited) or by the drug permeating across biological membranes *e.g.* the intestinal mucosa (permeation rate limited), *in vitro* models are commonly designed to simulate these processes (Dressman et al., 1998; Amidon et al., 1995; Berthelsen,

* Corresponding author.
E-mail addresses: sfv995@sund.ku.dk (M. Klitgaard), dlt527@alumni.ku.dk (P.J. Sassene), arzu.Selen@fda.hhs.gov (A. Selen), anette.mullertz@sund.ku.dk (A. Müllertz), ragna.berthelsen@sund.ku.dk (R. Berthelsen).

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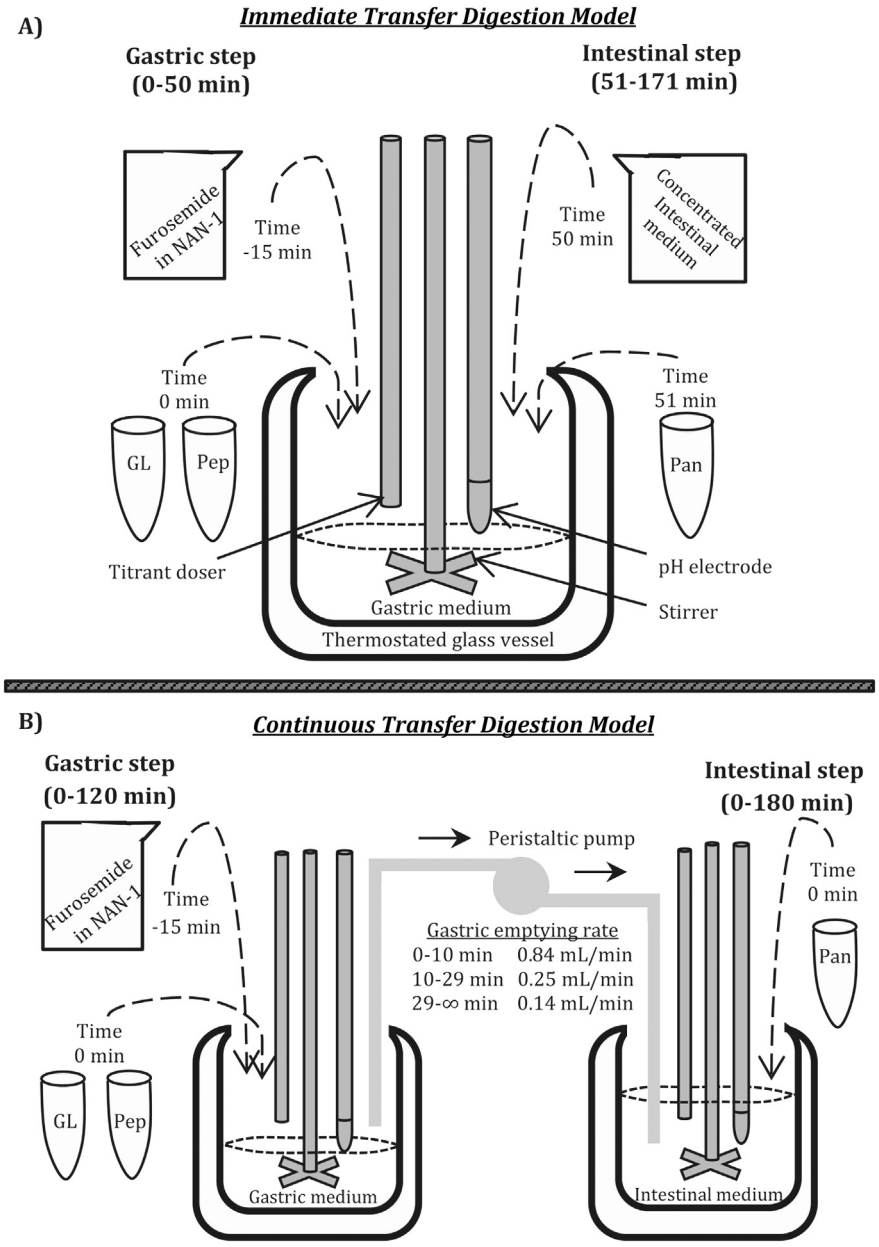

Fig. 1. Schematic overview of the immediate transfer (A), and the continuous transfer (B) *in vitro* digestion models. In the Immediate Transfer Digestion Model the addition of the digestive enzymes, as well as the change from the gastric to the intestinal step is time specific. In the Continuous Transfer Digestion Model, the transfer rate is varied over time simulating the gastric emptying, using a peristaltic pump. GL: gastric lipase, Pep: pepsin, Pan: pancreatic lipase.

2016). With respect to neonates (0–1 months) and infants (1–12 months) who are fed on a regular basis, the digestion of an ingested meal (breast milk or infant formula) will most likely impact the GI drug solubilization (Kamstrup et al., 2016). During intestinal digestion of the milk lipids, different colloid phases are formed, leading to the formation of mixed micelles consisting of bile salts, phospholipids, monoglycerides and free fatty acids (Porter et al., 2007). As the administered drug will be solubilized to a different degree in the aqueous -, colloid -, and lipid phases formed upon digestion, *in vitro* models used to predict drug absorption in neonates and young infants should simulate the GI digestion.

With the increased focus on drug delivery to the pediatric population, a few *in vitro* models have been designed to mimic drug dissolution and absorption in specific pediatric populations (Kamstrup et al., 2016; Havenaar et al., 2013; Bourlieu et al., 2014; Mooij et al., 2012). However, the number of studies involving pediatric *in vitro* models presently available in the literature is still very limited. Havenaar et al. (2013) have designed an *in vitro* model simulating the GI tract of pediatric patients in the age range 0–2 years split in to three categories; under term-neonates, infants and toddlers. The model is based on the computer-controlled, multi-compartmental *in vitro* GI system developed by TNO (TNO Intestinal Model, TIM) with a new set of specifications to mimic the kinetic conditions in the upper part of the GI tract in children (TIMPediatric) (Havenaar et al., 2013). Based on the *in vivo – in vitro* relations obtained using the original TIM-1, TIMPediatric inheritably shows promise. However, as the two models are based on the same setup, they are expected to share the same disadvantages such as high risk of drug adsorption to the many plastic surfaces and filters leading to a low recovery, as well as a very low throughput due to long run times and time consuming cleaning processes (Berthelsen, 2016). Aside from the TIMPediatric described by Havenaar et al. (2013), key aspects to consider when developing *in vitro* models simulating digestion and drug solubilization in pediatric populations have been discussed and published in several reviews (Kamstrup et al., 2016; Bourlieu et al., 2014; Mooij et al., 2012). Based on the available information on the physiology and feeding patterns of neonates and young infants (age 0–2 months), Kamstrup et al. (2016) suggested an *in vitro* model simulating the GI digestion in this pediatric population (neonates and