Studying the Oral Performance of Furosemide Using an In Vitro Model Simulating Gastro-Intestinal Digestion and Drug Solubilization in Neonates and Young Infants

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Purpose

In vitro models are a commonly used tool to estimate oral drug performance prior to conducting in vivo studies. The 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 gastro-intestinal (GI) fluids and by the drug permeating across biological membranes, in vitro models are commonly designed to simulate these processes. With respect to neonates and young infants who are fed on a regularly basis, the digestion of an ingested meal will most likely impact the drug solubilization, for which reason it should be incorporated into the in vitro models used to estimate drug absorption in this pediatric population. The aim of the present study was to predict the solubilization of furosemide using a newly developed in vitro model simulating the digestion and drug solubilization in the GI tract of neonates and young infants.

Methods

Drug solubilization was evaluated using an in vitro model simulating the gastrointestinal tract of the human neonate and young infant population (age 0-2 months). The model mimics the stomach and small intestine using physiologically relevant media, volumes and digestive enzymes.

All experiments were carried out following the same basic procedure. Furosemide was initially dispersed in infant formula (corresponding to a meal) for 15 min (in the fasted state studies the infant formula was replaced by a small volume of water). After dispersion, the drug suspension was transferred to a thermostated glass reaction vessel (37.5 °C) containing concentrated gastric media to produce a fed state simulated gastric media. Gastric digestion was initiated by addition of gastric lipase and pepsin. During gastric digestion, the pH in the reaction vessel was kept constant at 6.4 by automated addition of 0.5 M NaOH. At time 50 min, a fixed volume of concentrated intestinal medium was added and the pH adjusted to 6.5 by automated addition of 0.5 M NaOH, thereby, creating a fed state simulated intestinal medium. The intestinal digestion was initiated by addition of pancreatic lipase. Throughout the digestion experiment samples were taken from the reaction vessel at designated time points and the digestion was inhibited by addition of protease -, and lipase inhibitor. The samples were ultracentrifuged resulting in a phase separation of 3 phases: a lipid-rich insoluble phase, an aqueous phase and a pellet phase. The drug concentration in each phase was determined by HPLC. The study showed that the solubilization of furosemide was affected by the presence of food; i.e. almost the entire dose was solubilized in the aqueous phase during fed state gastric and intestinal digestion. Less than 3.0 % of the dose was solubilized in the aqueous phase during fasted state gastric digestion and only 60.7 ± 1.1 % of the dose during fasted state intestinal digestion.

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, it was possible to estimate the importance of these factors in vivo. In these studies the presence of food (food-effect) as well as the effect of digestion (tested with and without digestive enzymes) was evaluated. In all studies, the tested dose of furosemide was 3 mg/kg, as this is the recommended dose for oral administration to neonates and young infants.

Results

The model thoroughly.

The developed in vitro model was successfully used to estimate the impact of various effect of the solubilization of furosemide in the GI tract of neonates and young infants. The present in vitro data suggests that the oral performance of furosemide in neonates and young infants will be increased by presence of food (frequent feedings) due to solubilization but that it is not remarkably influenced by digestion of meals in the GI tract. Further studies including different model drugs and in vivo data are needed to evaluate the in vitro model thoroughly.