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Research paper

Simultaneous lipolysis/permeation *in vitro* model, for the estimation of bioavailability of lipid based drug delivery systems

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ABSTRACT

The simultaneous processes of lipid digestion and absorption together determine the oral bioavailability of drugs incorporated into lipid based drug delivery systems (LBDDS). A number of slightly different protocols for *in vitro* lipolysis are widely accepted; however, the permeation process has so far not been included into the models due to the harsh conditions of lipid digestion compromising permeation barriers. The present study for the first time combines biomimetic permeation and lipolysis of LBDDS.

The focus of the current work was on the functional stability of the barrier - Permeapad® during lipid digestion. Using calcein as a marker molecule the investigations demonstrated that the barrier was able to maintain its permeation properties in the presence of the SNEDDS (self-emulsifying drug delivery system) formulation, the lipolysis medium, and the lipolysis medium while digesting the SNEDDS. Furthermore, the permeation of cinnarizine (CINN) from SNEDDS was demonstrated to be lower, if the formulation as such was applied as compared to the digested formulation. This support the general perception that meaningful *in vitro* evaluation of lipid based formulations requires consideration of both, the digestion and absorption, i.e. lipolysis and permeation.

1. Introduction

Many of the newly developed chemical entities in the pharmaceutical industry have a high permeability, but a poor aqueous solubility, hence being classified as class II drugs in the biopharmaceutical classification system (BCS) [1]. Dependent upon the physical-chemical characteristics that make the compounds poorly aqueous soluble, they can be denoted as either "grease balls" or "brick dust" [2]. Brick dust are in general terms, used to describe compounds with both a high log P and a high melting point, whereas grease balls are highly lipophilic compounds (Log P > 4) with a low melting point (<100 °C). While the solubility of grease balls type materials in aqueous media is low, it is frequently observed that these compounds are highly soluble in lipids, though this rule of thumb does not apply in all cases [3]. Generally, this makes grease ball type molecules very suited for lipid based drug delivery systems (LBDDS) such as e.g. self-nano-emulsifying drug delivery systems (SNEDDS). For poorly aqueous soluble drugs, LBDDS offers the advantage over conventional dosage forms such as tablets and capsules to present the molecule in a pre-solubilized form into the intestine, thereby omitting the potential rate determining step for absorption – namely the dissolution of the compound. Once taken orally the lipids will be digested in

Abbreviations: ACN, acetonitrile; NaTC, sodium taurocholate; LBDDS, lipid based drug delivery system; SNEDDS, self-nano-emulsifying drug delivery systems; CINN, cinnarizine; PAMPA, parallel artificial membrane permeability assay; PVPA, phospholipid vesicle-based permeation assay; MCDK, Madin-Darby Canine Kidney; PBS, phosphate buffer saline; TG, triglycerides; FFA, free fatty acids.

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the gastro intestinal tract (GI-tract). The digestion of lipids will result in formation of surface active mono-glycerides and di-glycerides, and the drug will be liberated from its pre-solubilized form. Drug molecules solubilized inside these micelles and in other colloidal structures present in the gastro intestinal fluids first need to be released from such structures and become freely (truly) molecularly dissolved, before their uptake [4]. This fact is widely discussed to be the reason for poor predictability of bioavailability from apparent solubilities [5,6]. Therefore the digestion step of the LBDDS is very important for drug release and subsequently the absorption.

Different models have been suggested for simulating the intestinal lipid digestion *in vitro*. The lipid digestion process is facilitated by different pancreatic enzymes, such as co-lipase, dependent pancreatic lipase [7], which can be mimicked *in vitro* using an *in vitro* lipolysis model [8,9]. The pancreatic lipase hydrolyzes the triglycerides (TG) in a LBDDS into two free fatty acids (FFA) and one mono-glyceride, thereby allowing transfer of the dissolved drug into mixed intestinal micelles. The composition of *in vitro* lipolysis medium consists primarily of bile salts and phospholipids, often in the ratio of 4:1 [10]. Porcine pancreatin is often used as enzyme blend due to its similarity in enzymatic composition in human pancreatic fluid [11]. It is widely accepted, that release of a compound, solubilized in a LBDDS can be investigated in an *in vitro* lipid digestion model, and discussions about standardization is also prevalent [12]. It has been reported that the drug release of cinnarizine (CINN) from a SNEDDS formulation during digestion was high [13]. The precipitation of CINN observed *in vitro* was of limited importance since the *in vivo* data did not show any difference between the formulations, despite that some of the formulations lead to precipitation *in vitro* and others did not [14,15]. It has further been suggested that the precipitate, being amorphous, would obstruct absorption less than a crystalline precipitate, assuming that the compound has a significant absorption during its passage through the gastro intestine. Hofmann and coworker [16] suggested therefore a model combining *in vitro* lipolysis with *ex-vivo* intestinal permeation; however no correlation was obtained when compared to *in vivo* bioavailability data from rats [16]. More recently Porter and coworkers [17] have described an *in vitro* lipid digestion model coupled with a single pass *in situ* intestinal perfusion setup in order to better investigate and understand the interplay between drug solubilisation, precipitation and absorption [17]. However, this model is time-consuming and complicated, not to mention, the high variability, when using animals and the associated ethical considerations.

Common alternative permeation studies are cell-based studies, such as Caco-2 and Madin-Darby Canine Kidney (MDCK) [18,19], or the non-cell based studies, such as parallel artificial membrane permeability assay (PAMPA) and phospholipid vesicle-based permeation assay (PVPA) [20,21]. However, none of these methods has been demonstrated to be able to withstand the harsh conditions of the lipolysis medium and pancreatic enzymes, not to mention the different surfactants and solvents used in LBDDS and other enabling formulations. Permeapad® has in earlier studies shown good resistance to a number of surfactants and solvents [22]. An *in vitro* model combining lipolysis and permeation using the biomimetic barrier may be an approach to better understand the drug uptake from LBDDS. The model may provide formulation scientists in an early phase of development with better prediction tools of drug uptake, given the fact that the uptake/permeation of drug molecules will affect the equilibrium of solubilized and freely dissolved drug.

The aim of this study was, therefore to combine *in vitro* lipid digestion and permeability in a simplified experimental setup and to investigate the usability of Permeapad® for lipolysis/permeation studies, using a LBDDS containing CINN as the model drug.

2. Materials and methods

2.1. Materials

Brij® 97, calcein, calciumcloride dihydrate ($CaCl_2{\cdot}2\ H_20$), cinnarizine, Cremophor® RH 40, oleic acid, pancreatin from porcine, sesame oil, sodium taurocholate, trismaleate, and 4-bromobenzene boronic acid (4-BBA) were all obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Soy phosphatidylcholine (PC) S-100 was a generous gift from Lipoid GmbH (Ludwigshafen, Germany). Sodium dihydrogen phosphate dihydrate ($[NaH_2PO_4]{\cdot}2\ H_2O$), di-sodium hydrogen phosphate dodecahydrate ($[Na_2HPO_4]{\cdot}12H_2O$), maleic acid, sodium hydroxide (NaOH), hydrochloric acid (HCl) and sodium chloride (NaCl) used for the different buffers were all obtained from Sigma-Aldrich. All solvents used were of analytical grade and bought from Sigma-Aldrich. Water used for all the experiments was obtained from a MilliQ purification system.

2.2. Methods

2.2.1. Preparation of biomimetic barrier

The Permeapad® (Certificate No. 014557268) barrier was prepared as previously described by Di Cagno and Bauer-Brandl [23] using soy phosphatidylcholine S-100 as the lipid layer. In brief, a thin layer of lipid was applied to a hydrophilic support sheet (Pütz GmbH, Taunusstein, Germany). The lipid was dissolved in an organic solvent, which was allowed to evaporate after applying the organic solution to the support sheet, thereby forming the barrier. The final barrier therefore consisted of support layer and lipid layer. Alternatively the empty support sheet was used as a barrier. It is a hydrophilic polymer membrane (cellulose hydrate) which acts as a diffusion barrier. All barriers employed in this work were stored at room temperature protected against sunlight.

2.2.2. Preparation of the SNEDDS and lipolysis medium

The SNEDDS were prepared by weighing and mixing the components, see Table 1. Ethanol was added as the last excipient to minimize evaporation. The mixture was stirred until homogeneous. 50 mg/g cinnarizine was added to the mixture, and stirred until it was completely dissolved.

The lipolysis medium was prepared according to the protocol of [24], with some slight modifications, by dissolving the components listed in Table 2 in MilliQ water. The medium was prepared one day prior to the lipolysis experiments and was let to equilibrate at 37 °C until the next day, when pH and volume was finally adjusted.

Pancreatic extract solutions for the lipolysis experiments were prepared 10 min prior use to limit denaturation. The extract was prepared by gently vortexing pancreatic extract into Milli Q water until homogenously mixed. Enough pancreatic extract was added to obtain a lipase activity of 600 USP units (USP/mL). When

Table 1
Composition of the formulation and drug concentration used in the lipid based drug delivery system (SNEDDS).

SNEDDS composition	
Components	Ratio (w/w)%
Cremophor RH 40	45
Oleic acid	15.4
Brij 97	9
Sesame oil	20.6
Ethanol	10
Cinnarizine	50 mg/g