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Research Paper

Chain length affects pancreatic lipase activity and the extent and pH–time profile of triglyceride lipolysis

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ABSTRACT

Triglycerides (TG) are one of the most common excipients used in oral lipid-based formulations. The chain length of the TG plays an important role in the oral bioavailability of the co-administered drug. Fatty acid (FA) chain-length specificity of porcine pancreatic lipase was studied by means of an *in vitro* lipolysis model under bio-relevant conditions at pH 6.80. In order to determine the total extent of lipolysis, back-titration experiments at pH 11.50 were performed. Results suggest that there is a specific chain length range (C2–C8) for which pancreatic lipase shows higher activity. This specificity could result from a combination of physicochemical properties of TGs, 2-monoglycerides (2-MGs) and FAs, namely the droplet size of the TGs, the solubility of 2-MGs within mixed micelles, and the relative stability of the FAs as leaving groups in the hydrolysis reaction. During experimentation, it was evident that an optimisation of lipolysis conditions was needed for tighter control over pH levels so as to better mimic *in vivo* conditions. 1 M NaOH, 3.5 mL/min maximum dosing rate, and 3 μL/min minimum dosing rate were the optimised set of conditions that allowed better pH control, as well as the differentiation of the lipolysis of different lipid loads.

1. Introduction

Since the advent of high throughput techniques and development of combinatorial chemistry in the early 1990s, the number of potential drug candidates has significantly increased [1]. Physical properties of the new chemical entities have changed towards higher molecular weight, higher melting point, increased H-bonding capacity, and increased lipophilicity, leading to poorer solubility in aqueous media [2]. Indeed, it was estimated that in 2005 40% of the top 200 oral marketed oral drugs were poorly water-soluble [3]. Latterly in 2007, it was reported that up to 70% of the new active molecules in the development pipeline exhibited poor aqueous solubility [4]. Since low aqueous solubility can be associated with poor absorption and hence poor bioavailability, it is clear that one of the main challenges for pharmaceutical scientists is finding novel formulations capable of improving the intraluminal solubility of poorly soluble drugs.

The co-administration of hydrophobic drugs with dietary or formulation lipids in many cases results in improved oral bioavailability. Different proposed mechanisms by which lipidic formulations increase oral bioavailability include the following: (a) promoting drug solubilisation in the gastrointestinal tract, by providing lipidic components that increase the inherent solubilisation capacity of the intestinal fluids [5], (b) delaying gastric emptying and transit time [6], (c) increasing apparent drug permeability through inhibition of efflux transporters such as P-glycoprotein [7,8], (d) changing the membrane fluidity of enterocytes [9], and (e) reducing hepatic first-pass metabolism if lymphatic transport is involved [10].

Recently, there has been a growing interest in oral lipid-based drug delivery systems (LBDDSs) as a formulation strategy for efficient delivery of poorly water-soluble compounds [11,12–14]. Marketed formulations such as Marinol® (dronabinol) [15],

Abbreviations: DLS, dynamic light scattering; FA, fatty acid; LBDDS, lipid based drug delivery system; LCT, long-chain triglyceride; MCT, medium-chain triglyceride; MG, monoglyceride; SCT, short-chain triglyceride; TBU, tributyrin unit; TG, triglyceride; Tri-C2, glyceryl triacetate; Tri-C4, glyceryl tributyrate; Tri-C8, glyceryl trioctanoate; Tri-C10, glyceryl tridecanoate; Tri-C18, peanut oil.

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Neoral® (cyclosporine A) [16], Rocaltrol® (calcitriol) [17], Agenerase® (amprenavir) [18] and Accutane® (isotretinoin), have demonstrated that LBDDSs are an accepted and successful commercially viable formulation strategy for sparingly soluble compounds.

In vitro lipolysis is capable of mimicking the intestinal lipid digestion process, and therefore is a suitable method to trace the fate of drugs delivered by means of LBDDSs. The *in vitro* lipolysis model (extensively reviewed elsewhere [19–21]) has been previously developed and utilised by different research groups (including the University of Copenhagen [22,23], Monash University [24], Gattefossé [25] and The Hebrew University of Jerusalem [26]). Although the concept and fundamental principles of the model are similar between groups, experimental conditions and parameters vary among them. In this regard, the Lipid Formulation Classification System Consortium has published a number of studies aimed to reduce the variability in the experimental approach between different groups [7,27,28]. Briefly, the protocol for *in vitro* lipolysis consists of the dispersion of the LBDDS in the experimental medium consisting of simulated intestinal fluids. The addition of pancreatic lipase to the medium initiates the lipolysis process. The digestive enzyme hydrolyses triglycerides (TGs) in the formulation, releasing fatty acids (FAs) and inducing a drop in pH. In order to keep the pH at a constant value throughout the experiment (to mimic *in vivo* conditions), a pH-stat titrator is used. The instrument continuously measures and controls this transient drop in pH by equimolar titration of NaOH. The University of Copenhagen has used a slightly different approach, a dynamic *in vitro* lipolysis model, in which the rate of hydrolysis is controlled by continuous addition of calcium chloride [22,23]. Once the process is finished (or deliberately stopped by addition of an inhibitor), the resulting solution is ultracentrifuged and separated into three distinct layers: (i) an upper undigested lipid phase, (ii) a middle aqueous phase, containing colloidal structures within which poorly-water soluble drug molecules are solubilised, and (iii) a lower sediment phase, comprising FAs calcium soaps. It is assumed that drug molecules solubilised in the aqueous micellar phase are most readily available for absorption. After density-gradient separation, each phase is analysed for drug content. Finally, the percentage of drug dose solubilised in the aqueous phase *in vitro* is then compared with the *in vivo* pharmacokinetic data obtained following oral administration (to an animal or a human) of the LBDDS.

TGs are the main constituents of dietary lipids [29] and one of the most common excipients used in LBDDSs [12]. TG-based drug delivery systems, which belong to Type I formulations according to Lipid Formulation Classification System [30], are the most basic LBDDSs since they include neither surfactants nor co-solvents. The FA chain length of the TG in the formulation is an important factor in the oral bioavailability of the co-administered poorly water-soluble drug [31]. In general, following absorption into the enterocyte, lipolysis products derived from short-chain TGs (SCTs, <C6) and medium-chain TGs (MCTs, C6–C12) diffuse across the cell gaining access to the portal vein. However, FAs and MGs derived from long-chain triglycerides (LCTs, >C12) are re-esterified, incorporated into chylomicrons, and enter the lymphatic system, bypassing the hepatic first-pass metabolism [32]. SCTs are known to induce tight junction permeability changes [33], while the micellar solubilisation capacity of MCTs and LCTs has been proven to be higher than that of SCTs [26,34]. Although the assessment of the performance of TGs with different chain lengths has been carried out before, these studies have only focused on the end result, i.e. drug solubilisation across lipolysis phases [7,24,26,35–37]. Limited attention has been drawn to the causes for substrate specificity of the pancreatic lipase [38,39]. A better knowledge of the lipolysis process itself, and the factors governing lipase activity, would help to rationalise the performance of LBDDSs and eventually aid in the development of optimised lipidic formulations.

Accordingly, the first objective of this study was to gain a deeper understanding of the mechanism behind pancreatic lipase activity, by evaluating the *in vitro* lipolysis of equimolar amounts of TGs with different chain lengths.

Because different pH–time profiles were observed during the lipolysis of TGs with different chain lengths, it was evident that an optimisation of lipolysis conditions was needed for tighter control over pH levels so as to better mimic *in vivo* conditions. Therefore, the second aim of the study was to find an optimised set of conditions (in terms of titrant concentration and maximum and minimum titrant addition rates) capable of maintaining the pH environment within the physiological range (6.75–6.85) during the hydrolysis of TGs with different carbon chain lengths. The hydrolysis of different volumes of oil was also evaluated to assess a variety of possible scenarios in the intestine, from the ingestion of an oil-containing capsule in fasting conditions to the consumption of a high-fat meal.

2. Materials and methods

2.1. Materials

Sodium hydroxide solutions (NaOH, 0.5 M and 1 M), Trizma® maleate, sodium taurocholate hydrate (98% w/w), L-α-lecithin (~60% pure L-α-phosphatidylcholine, from egg yolk), pancreatin powder from porcine pancreas (8 × United States Pharmacopeia specifications activity), glyceryl triacetate (≥99.9%), glyceryl trioctanoate (≥99%), and peanut oil were all purchased from Sigma–Aldrich (Dorset, UK). Sodium chloride (99.5% w/w) was a product from Fisher Scientific (Leicester, UK). Calcium chloride anhydrous (93% w/w), and glyceryl tributyrate (98%) were purchased from Alfa Aesar (Heysham, UK). Glyceryl tridecanoate (≥98%) was obtained from TCI (Tokyo, Japan). The standard buffer solutions (pH 4, 7, 10 and 12), utilised for calibration of the pH-electrode, were purchased from YSI Incorporated (Ohio, USA) and Hanna Instruments (Rhode Island, USA). Water was obtained from a Purelab Ultra Genetic purification system (Elga LabWater, Illinois, USA).

2.2. Lipidic formulations

Glyceryl triacetate (tri-C2) and glyceryl tributyrate (tri-C4) served as model molecules for SCTs (<C6). Glyceryl trioctanoate (tri-C8) and glyceryl tridecanoate (tri-C10) represented MCTs (C6–C12). In a similar manner to previous publications [26,40], peanut oil (tri-C18) was chosen as the prototype for LCTs (>C12). Peanut oil contains mainly LCTs (C16 and C18), the vast majority of which is triolein [29].

2.3. Preparation of simulated digestion buffers

The preparation of the bio-relevant digestion buffer simulating the contents of the jejunum in the fasted state was based on previous reports [26,41] with a minor modification. This change consisted in decreasing the pH of the buffer from 7.40 to 6.80 to achieve maximum pseudo-physiological conditions [18]. The lipolysis medium contained 50 mM Trizma® maleate [35,36,42,43], 150 mM sodium chloride, 5 mM calcium chloride, 5 mM sodium taurocholate, and 1.25 mM L-α-lecithin. The pH of the medium was adjusted to 6.80 ± 0.05 at 37 °C using 1 M NaOH solution as titrant, and a pH-stat titrator unit (T50 Graphix, Mettler Toledo Inc., Leicester, UK) coupled to a pH-electrode (DGi111-SC, Mettler Toledo Inc., Leicester, UK).