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Research paper

Characterization of fasted human gastric fluid for relevant rheological parameters and gastric lipase activities

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Purpose: To characterize human gastric fluid with regard to rheological properties and gastric lipase activity. In addition, traditional physicochemical properties were determined.
Methods: Fasted HGA were collected from 19 healthy volunteers during a gastroscopic examination. Rheological characterization of the aspirates was conducted on a TA AR-G2 rheometer, using cone and plate geometry. Lipase activity was measured by continuous titration of released free fatty acid from tributyrate. Further, pH, osmolality, buffer capacity, and surface tension were measured and the total protein content and bile salt level were determined using assay kits.
Results: Rheological examination of HGA showed non-Newtonian shear-thinning behavior with predominant elastic behavior in the linear range. The apparent viscosity was measured to be in the range of 1.7–9.3 mPa s at a shear rate of 50 s^{-1}. The FaSSGF and HCl pH 1.2 have no shear-thinning properties and showed lower viscosity (1.1 mPa s at 50 s^{-1}). The observed viscosity of the HGA will decrease the intrinsic dissolution rate of drugs. The activity of the gastric lipase was 7.4 ± 4.0 U/mL ($N = 6$, $n = 3$) and 99.0 ± 45.3 U/mL ($N = 19$, $n = 3$) at pH 2.8 and 5.4, respectively. pH, surface tension, buffer capacity, bile salt concentration, and osmolality were measured and compared with literature data.
Conclusion: The rheological behavior and the mean apparent viscosity of HGA are significantly different from that of water and should therefore be considered important during development of gastric simulated media. Further, the activity of the HGL is active even under fasted gastric conditions and might contribute to the digestion and emulsification of lipid-based drug delivery systems in the entire gastrointestinal tract. HGL should therefore be considered in gastric evaluation of lipid-based drug delivery systems.

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1. Introduction

The *in vivo* performances of oral dosage forms are dependent on the physicochemical characteristics of the drug and of the type and characteristics of the dosage form, as well as of the physiological environment in the gastrointestinal tract. Factors such as pH, surface tension and osmolality have therefore been tested in the physiological environments in order to establish knowledge that enables development of media that mimics physiological conditions [1–3].

In order to assess dosage form performance in the stomach, simulated gastric media are often employed. These media represent a simplification of the luminal fluids [4–7]. The most simple dissolution media simulating gastric fluid is the media used in the USP method. This medium consists of 0.1 M HCl and has a surface tension of 68 mN/m. Vertzoni et al. developed a fasted gastric media, FaSSGF, containing *in vivo* relevant pH, surface tension, pepsin level, and a low level of taurocholate and phospholipids. FaSSGF was found to give a better prediction of the intraluminal dissolution of a lipophilic weak base (GR253035X), but not of a non-ionizable drug (atovaquone) [8]. Erceg et al. increased the osmolarity of FaSSGF to a more biorelevant level by increasing the NaCL concentration from 34 mM to 68 mM and thereby created FaSSGF-V2 [8–10]. Dissolution in the existing fasted simulated gastric media do not constitute the best possible reflection of the *in vivo* situation, because they do not take into account some physiological characteristics, such as presence of gastric lipase, mucin, and viscosity, which possibly can affect drug dissolution characteristics [8]. The rheological properties of human gastric fluid (HGF) have not been considered when developing fasted state biorelevant dissolution media (BDM). Mucus is present in fasted HGF. Mucus consists of mostly water and of entangled mucins and other

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mucus components with reversible linkages [11]. The mucin molecules are glycoproteins of high molecular weight, $2–14 \times 10^6$ g/mol [12]. The mucin molecules are to a large extent responsible for the viscosity and gel-forming properties of the mucus [13]. A number of mucins are present in the human gastrointestinal mucus and are either membrane bound (MUC 1 and MUC 4) or secreted (MUC2, MUC5AC, MUC5B, and MUC7) [14]. Further mucus consists of DNA, lipids, ions, proteins, cells, cellular debris, and water [11]. HGF has a higher viscosity than water or FaSSGF due to the presence of mucus, proteins, and lipid components [14]. The Noyes Whitney equation predicts that an increased viscosity decreases the dissolution rate of a drug [15]. Further, it has been shown that an increased media viscosity significantly delay tablet disintegration [16–18]. Therefore, the disintegration and dissolution rate of drugs and dosage forms both *in vivo* and *in vitro* is expected to decrease. The viscosity of the luminal content can have an impact on wettability of the drug and thereby on the dissolution of the drug in the luminal fluids, through its effect on diffusivity, mixing, and flow patterns in the gut [19].

The stomach secretes two enzymes important for nutrient digestion; gastric lipases and pepsin [20,21]. Not much focus has been on the existence of the human gastric lipase (HGL) in the fasted human stomach and the importance in drug dissolution and bioavailability when ingesting a drug in a lipid-based drug delivery system. HGL is present in the fasted stomach, and the basal level of HGL is about 0.1 mg/mL [22]. HGL has not been considered important when simulating the fasted gastric conditions and the developed fasted gastric simulated media (FaSSGF) contain pepsin and not HGL [8]. It has been shown that the lipolysis of lipids begins in the stomach catalyzed by HGL. HGL is secreted by chief cells located in the fundic mucosa and is active and stable at acidic pH [23,24]. HGL is a highly glycosylated globular protein with a molecular weight of 50 kDa and has the highest activity toward triglycerides at pH 5.4–6.0, but it has shown to be active in the pH range 2–8 [25,26]. Carriere et al. found that triglycerides are digested to one diglyceride (DG) and one free fatty acid (FFA) in the stomach [27]. The residence time in the stomach has been measured to be approximately 40 min and 30 min for medium chain triglycerides formulation and for a placebo formulation, respectively [28]. Gastric lipolysis accounts for 10–25% of ingested triglycerides and may therefore have a high impact on drug bioavailability when ingested together with food or as a lipid-based drug delivery system even in fasted state [27,29]. Studies by Diakidou et al. in the fed state showed that HGL significantly impacts the solubility of weak bases (dipyridamole, ketoconazole) and increases the release of the weak base, felodipine from HPMC matrices [30,31].

In a study by Devle et al., it was shown that human gastric and intestinal enzymes changed the viscosity of the gastric content during digestion of two types of milk products. Therefore, it is expected that the gastrointestinal enzymes might not only directly impact drug dissolution by digestion and solubilization but also by changing the viscosity of the gastric content after ingestion of food [32].

The purpose of this study is to extend characterization of fasted HGF, with an assessment of rheological properties as well as gastric lipase activity, in addition to the traditional physicochemical parameters. Obtained data are compared with literature values and the traditionally used dissolution media FaSSGF and HCL (pH 1.2).

2. Materials and methods

2.1. Materials

Sodium chloride (NaCl), calcium chloride dihydrate ($CaCl_2$), and sodium hydroxide pellets (NaOH) were all purchased from Merck (Damstadt, Germany). Pepsin from porcine gastric mucosa, Taurocholic acid sodium salt hydrate (NaTC), albumin from bovine serum, and glycerol tributyrate were all purchased from Sigma–Aldrich (Saint Louis, MO, USA). Phosphatidyl choline (Lipoid S PC) was purchased from Lipoid (Ludwigshafen, Germany). Purified water was obtained from a Millipore Milli-Q Ultrapure Water Purification System (Billeria, MA, USA).

2.2. Volunteers for the study

Human gastric aspirates (HGA) were collected from volunteers during gastroscopic examinations at Gentofte Hospital, Copenhagen. Nineteen volunteers with normal body weight, aged 20–79 years old, were included in the study. The volunteers were not allowed to eat or drink, 6 and 2 h prior to the examination, respectively. Only volunteers that did not have any upper gastrointestinal diseases were included. Smokers, pregnant or breastfeeding women, and volunteers that ingested any medication, food or water on the day of gastric fluid aspiration were excluded. A few volunteers were excluded due to lack of compliance with the protocol, either due to diseases in the upper gastrointestinal tract, or because it was not possible to collect sufficiently amount of HGA for analysis. The volunteers all gave their written informed consent to the experimental procedure. The study was approved by the Ethical Committee of Denmark, Copenhagen, Denmark, and followed the convictions of the Declaration of Helsinki (H-2-2011-073).

2.3. Handling of samples

The aspirates were collected in volunteers immediately after introduction of a gastroscope. The volunteers were sedated with Propofol or Midazolam during the procedure. The aspiration was performed using a conventional gastroscope which has a build in suction mechanism. The gastroscopes used during the procedures were equipped with a camera and had a diameter ranging between 9 and 11 mm. The gastroscope was passed through the mouth and the esophagus into the stomach from where visible the gastric fluid was aspirated. No fluid was used to rinse the gastroscope prior the examination, and great care was taken not to flush the endoscope with water before the aspiration of gastric juice was performed. Immediately after collection, the samples were kept on ice. For the lipase activity measurement, one mL of each sample of HGA was immediately inhibited by protease inhibitors (final concentration benzenesulfonyl fluoride 1 mM, Aprotinin 0.8 μM, Bestatin 40 μM, E-64 14 μM, Leupeptin 20 μM, and Pepstatin A, 15 mM) to prevent proteolytic inactivation from occurring and then kept on ice.

The non-inhibited HGA samples were whirl mixed and analyzed for pH, osmolality, surface tension, and rheological characteristics. Thereafter, the samples were kept at −20 °C. Later, the samples were thawed and whirl mixed before measuring the buffer capacity, total bile acids concentration, and protein concentration.

2.4. Preparation of media, FaSSGF

Simulated gastric media, FaSSGF, was prepared according to Vertzoni et al. [8]. The concentrations of the components are given in Table 1. The solution was stirred overnight and pH was adjusted to 1.6 with HCL. Purified water was added to a final volume of 1000 mL, and the FaSSGF media was stored at 5° until used.

2.5. Physicochemical assays

2.5.1. pH

The pH values were measured by a pH electrode Metrohm (Switzerland) connected to a PHM 220 pH-meter (Radiometer, Denmark).

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