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Research paper

Simulation of fasting gastric conditions and its importance for the in vivo dissolution of lipophilic compounds

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Abstract

In this study, the importance of accurate simulation of fasting gastric environment for the assessment of the absorption process of two model lipophilic compounds, GR253035X (weak base) and atovaquone (non-ionizable), was assessed. Dissolution profiles were constructed in previously proposed simulated gastric fluids and in a new medium that comprises only of components that have been recovered from the fasting stomach. Dissolution data obtained in a more physiologically relevant medium led to better correlation between the simulated and actual intralumenal dissolution vs. time profiles for GR253035X. In contrast, accurate simulation of gastric environment did not affect the simulated plasma profile of atovaquone. Accurate simulation of the fasting gastric contents may be crucial for the assessment of the absorption profile of lipophilic weak bases.

Keywords: Dissolution; Fasted state; Gastric composition; GR253035X; Atovaquone; Weak base

1. Introduction

Media proposed to date for assessing dissolution in the fasting gastric contents do not constitute the best possible reflection of the in vivo situation, because they do not contain certain physiological substances that may affect dissolution characteristics, such as pepsin [1], they contain substances which are not physiologically relevant, such as artificial surfactants [2,3], or, they contain substances, such as pepsin or bile salts, at non-physiologically relevant concentrations [4,5]. Therefore, in order to screen for reliable and reproducible performance of dosage forms under gastric conditions, it would be highly desirable to develop gastric media which more adequately reflect physiological conditions [6].

For highly soluble compounds, dissolution during gastric residence is not important to the absorption profile regardless of dosing conditions (e.g. [7]). Similarly, dissolution during residence in the fasting stomach does not seem to be important for lipophilic compounds with weakly acidic characteristics (pK_a ~3.5–5.5), because dissolution in stomach contributes little to the overall dissolution whereas the pH conditions in the small intestine favor fast and often complete dissolution [8,9]. For lipophilic weak bases or non-ionizable compounds, there are no direct data showing the importance of dissolution during gastric residence on the absorption profile. Opposite to weak acids, weak bases dosed in the fasted state are expected to be primarily dissolved during residence in stomach (e.g. [10]). For non-ionizable compounds, in vitro data suggest that, although the extent of dissolution is higher in the fasted small intestine, the difference from fasting stomach is not dramatic (e.g. [11]).

The objectives of this study were twofold. The first was to prepare a medium that reflects the actual gastric composition in the fasting state according to published physiological data. The second was to address the importance of adequate

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simulation of gastric contents on the absorption vs. time profile of a lipophilic weak base (GR253035X) and a lipophilic non-ionizable compound (atovaquone).

2. Materials and methods

2.1. Materials

Pepsin from porcine stomach mucosa (0.064 mg pepsin/mg solid), taurocholic acid (sodium salt, >99% pure), Triton X-100, and sodium lauryl sulfate were purchased from Sigma Chemical, St Louis, USA. Egg-lecithin (Lipoid E PC, >98% phosphatidylcholine) was from Lipoid GmbH, Ludwigshafen, Germany. All other chemicals were of analytical grade.

Immediate release (IR) tablets of GR253025X (100 mg/tablet, Batch F97/005B) and atovaquone (Wellvone®, 250 mg/tablet, lot E96L1596) were donated from GSK, UK. Physicochemical characteristics of GR253025X ($C_{22}H_{17}N_2O_3FS$) and atovaquone have been summarized previously [12].

2.2. Dissolution tests

2.2.1. The in vitro setup

Dissolution experiments of GR253035X and atovaquone tablets were run in triplicate at 37 ± 0.5 °C using the USP II Apparatus (Distek® dissolution tester, model 2100B, North Brunswick, NJ, USA) with the paddle rotating at 100 rpm.

2.2.2. Volume of dissolution medium

In the fasted state, resting volumes are quite low, and have been estimated to be about 25 ml [13,14]. However, when a tablet or capsule is administered, some fluid is usually co-administered. In pharmacokinetic studies, this volume is often in the 200–250 ml range. Assuming secretions at a rate of just under 1 ml/min [15], about 50 ml secretions are expected within 1 h, the longest period during which a fast disintegrating immediate release dosage form is expected to be totally emptied from the fasted stomach [16]. Thus, a realistic volume to simulate the total fluid available in stomach to dissolve simple dosage forms during gastric residence that empty with the fluid after administration in the fasted state would fall in the range of 250–300 ml. It is of note that in the USP apparatus II (rotating paddle), often used for dissolution testing of immediate release dosage forms, the minimum volume that can be used is slightly more than 300 ml. Otherwise, the paddle is not completely immersed in the dissolution medium. Based on the above the volume of dissolution medium in the current studies was set at 500 ml.

2.2.3. Simulation of gastric composition in the fasted state

The median pH of the stomach in the fasted state has been usually reported to lie in the range 1.5–1.9 [17–19], although a few subjects may have pH values as low as below pH 1 or as high as pH 5–6 [17]. Basal pepsin output is about 0.8 mg/ml [20,21]. Therefore, assuming a resting volume of about 25 ml [13,14], in the fasting stomach the total amount of pepsin will be about 20 mg. Ingestion of a glass of water (about 200–250 ml) will bring the pepsin concentration at the time of administration down to about 0.08 mg/ml. Since the stomach empties faster than the rate at which pepsin is secreted, the concentration of pepsin during the first hour post-dosing is expected to slowly increase towards an upper limit of 0.8 mg/ml (corresponding to the time when no ingested fluid remains in the stomach). With regard to gastric lipase contents data suggest that basal levels are about 0.1 mg/ml [22]. Taking into account the dilution effect and the fact that gastric lipase is active at pH values between 3 and 6, the presence of this enzyme is unlikely to be important to drug dissolution in the fasting state.

Perhaps in the most thorough investigation of bile salt levels in the stomach, Rhodes et al. [23] radiolabeled the bile salt pool and used this technique to evaluate bile salt reflux into the stomach in the fasted and fed states. They found an average concentration of 80 μM total bile salts in the stomach in 10 healthy volunteers in the fasted state, with a standard error of 30 μM. These values are more than two orders of magnitude lower than concentrations in the small intestine in the fasted state. In later studies using diagnostic kits to measure the bile salt levels, Lindahl et al. [24] found low levels of bile salts in 16 of 36 aspirates from healthy volunteers (in 20 aspirates, no detectable levels could be found), resulting in a median concentration of 100 μM, while in four of eight volunteers studied by Efentakis and Dressman [19] the average value was 275 μM, noting that similar to the Lindahl study, no bile salts were detected in the gastric aspirates of the other four of the subjects. Taken together, these results indicate that bile salt reflux into the stomach occurs sporadically and that concentrations in the stomach are very low compared with those in the small intestine.

The surface tension of gastric aspirates has been measured by several groups. Finholt and Petersen [25] published values of 36–51 mN/m in gastric juice from nine healthy subjects. Similarly, Efentakis and Dressman [19] reported values of 35–45 mN/m in eight subjects and Pedersen et al. [26] reported values of 28–42 mN/m in five healthy subjects. It has been shown that observed concentrations of bile salts in the gastric aspirates cannot be solely responsible for the low surface tensions that are invariably observed in gastric aspirates [27]. Fig. 1 shows that addition of physiologically relevant concentrations of pepsin in HCl solution having a pH of 1.6 and containing 2 g/l NaCl reduces the surface tension to, about, 57 mN/m. Upon addition of physiologically relevant concentrations of NaTC and phosphatidylcholine (molar ratio 4:1 [2]) the values fell further to, about, 40 mN/m (Fig. 1). The significant surface tension lowering effect of minute amounts of bile salts and phosphatidylcholine is in

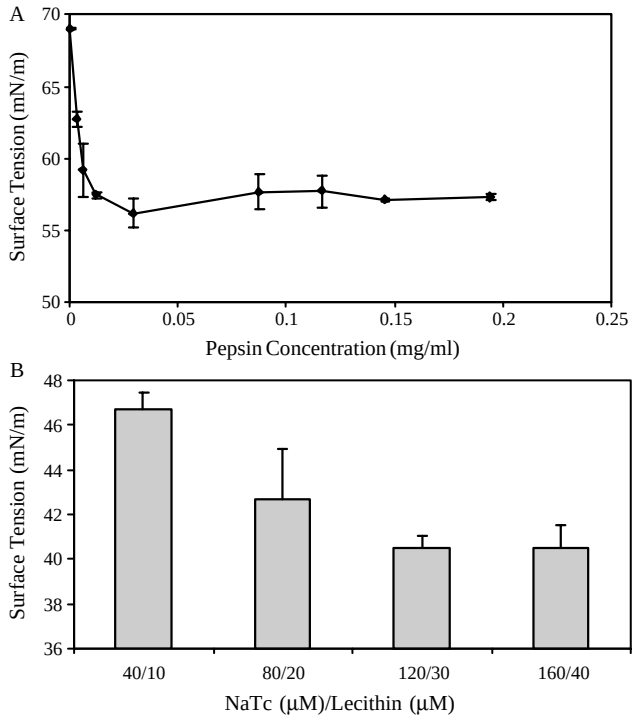

Fig. 1. (A) Surface tension of a 2 g/l NaCl solution having a pH 1.6 (with HCl) and containing various concentrations of pepsin. (B) Surface tension of a 2 g/l NaCl solution having a pH 1.6 (with HCl) containing 0.1 mg/ml pepsin and various concentrations of sodium taurocholate and phosphatidylcholine. In all cases, surface tension was measured in triplicate with the Du Noüy ring method at 37 °C. (Tensiometer Krüss Germany Nr 02276 equipped with a waterbath Valka TP100).

agreement with similar data published by Luner and VanDer Kamp [5].

The osmolarity of gastric juice in 36 gastric aspirates was reported to average 191 mosm/kg by Lindahl et al. [24] and these results were consistent with the data of Pedersen et al. [26] who reported values slightly above 200 mosm/kg. The principal cations in gastric juice are sodium (about 70 mM) and potassium (about 15 mM), while the principal anion is chloride (about 100 mM). Multivalent ions such as calcium are present in sub-millimolar concentrations, resulting in an ionic strength of about 0.1.

Based on the above, a fasted state simulating gastric fluid (FaSSGF) was designed (Table 1) and compared against two of the most frequently used 'simulated gastric fluids', i.e. USP's simulated gastric fluid without pepsin but containing sodium lauryl sulfate (anionic surfactant), SGF_{SLS} [2], or Triton X-100 (non-ionic surfactant), SGF_{Triton} [3] (Table 1).

2.2.4. Sample treatment and assay methods

Samples were drawn (with sample replacement) using a 5 ml Fortuna Optima syringe fitted with a stainless tubing. Samples were filtered through Titan® membrane filters (regenerated cellulose, 0.45 μm, Scientific Resources Inc., Eatontown, NJ, USA), discarding the first 1 ml. The adsorption of the drug onto the filter was checked and confirmed to be negligible. Assay of GR253035X and atovaquone was performed using a previously described method [12].

Table 1
Composition and physicochemical properties of the dissolution media used in the present study and of gastric contents

Physicochemical properties	SGF_{SLS}	SGF_{Triton}	FaSSGF	In vivo data [17,20,23,24]
Sodium lauryl sulfate (%, w/v)	0.25	–	–	–
TritonX100 (%, w/v)	–	0.1	–	–
Pepsin (mg/ml)	–	–	0.1	~0.8
NaTc (μM)	–	–	80	~80
Lecithin (μM)	–	–	20	–
NaCl (mM)	34.2	34.2	34.2	68±29
Surface tension (mN/m)	33.7	32.0	42.6	41.0±6.0
Osmolarity (mosm/kg)[a]	180.5±3.6	157.7±2.9	120.7±2.5	191±36
pH	1.2	1.2	1.6	1.4–2.1

[a] Measured with a semi-micro osmometer (Model A0300, Knauer GmbH, Berlin, Germany).

2.2.5. Data treatment

A model based on the theoretical physicochemical aspects of dissolution [28] was fitted to all dissolution data in SGF_{SLS}, SGF_{Triton}, FaSSGF and, also, to data in fasted state simulating intestinal fluid (FaSSIF) obtained previously [12].

2.3. In vitro–in vivo correlations

For GR252035X, cumulative-amount-dissolved-intralumenally vs. time plots were simulated using Stella 5.0 (Cognitus Ltd, North Yorkshire, UK) and a procedure published previously [28]. This procedure utilizes dissolution data under simulated fasted gastric conditions (obtained in the present study), population gastric emptying data, dissolution data obtained under simulated fasted intestinal conditions (obtained from [12]) and rapid uptake by the intestinal mucosa (i.e. no precipitation considerations upon arrival in the duodenum). Simulated plots were contrasted with plots constructed after deconvolution (WinNonlin 3.1, Pharsight, Mountain View, CA) of actual in vivo data. In vivo data had been collected after administration of a tablet (100 mg/tablet) and an oral solution (100 mg/dose) of GR253035X to 12 healthy fasted humans on a crossover basis (GSK data in file). Mean in vivo data sets were used to estimate the actual amount dissolved intralumenally vs. time plots.

For atovaquone, simulated plasma profiles were constructed using dissolution data in SGF_{SLS}, SGF_{Triton}, and FaSSGF (obtained in this study), data in FaSSIF [12] and the previously published procedure [28]. These profiles were contrasted with median actual data and the simulated profile obtained using data in water and FaSSIF [28].

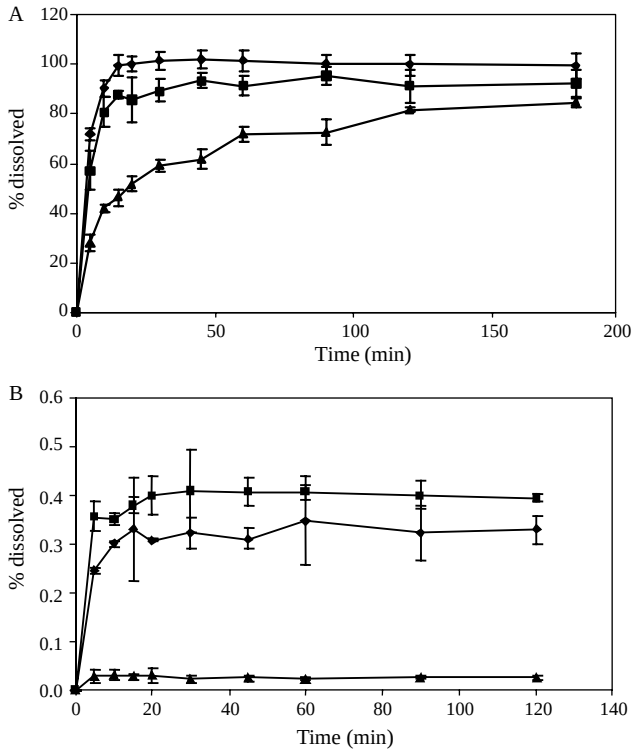

Fig. 2. Cumulative % dissolution data for GR253035X tablets (A) and atovaquone tablets (B) in SGF_{SLS} (♦), SGF_{Triton} (■), FaSSGF (▲).

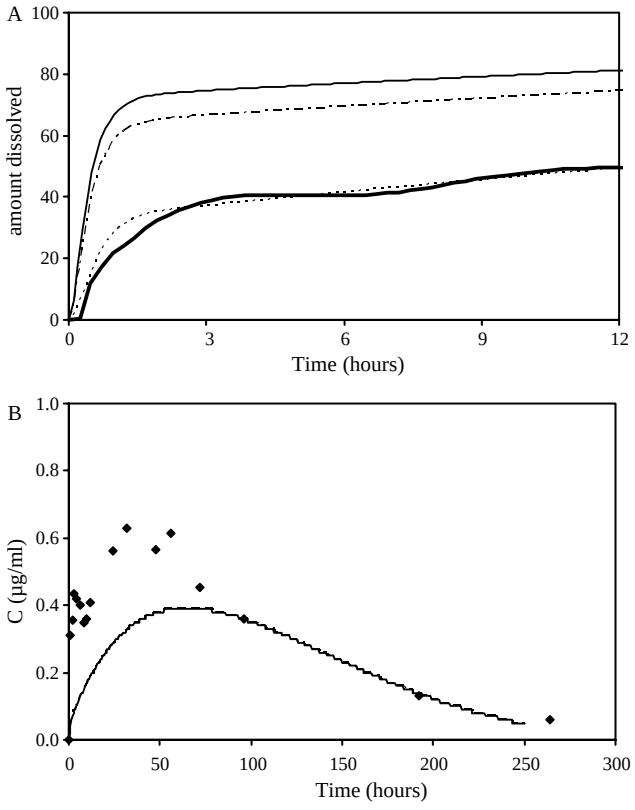

Fig. 3. (A) Actual cumulative amount of GR253035X dissolved intralumenally after the administration of one GR253035X tablet in the fasted state vs. time (——) and simulated cumulative amounts dissolved intralumenally vs. time plots using data in SGF_{SLS} and FaSSIF [12] (—), SGF_{Triton} and FaSSIF (– ·– ·– ·), and FaSSGF and FaSSIF (···). (B) Median observed plasma data after administration of one atovaquone tablet in the fasted state (♦) [11] and simulated plasma profiles using dissolution data in water and FaSSIF [28] or FaSSGF and FaSSIF or SGF_{SLS} and FaSSIF or SGF_{Triton} and FaSSIF (—).

3. Results

Fig. 2A shows that dissolution of GR253035X is slower in FaSSGF than in SGF_{Triton} or in SGF_{SLS}. As confirmed by separate sets of experiments in hydrochloric acid solutions having pH 1.2 and 1.6 (data not shown), this was mainly due to the pH differences between FaSSGF and SGF_{SLS} or SGF_{Triton}. For atovaquone, dissolution is very limited in all three media (Fig. 2B) with the profile in FaSSGF being similar to the profile in water [11].

Fig. 3A shows that dissolution data in FaSSGF lead to better prediction of the actual cumulative dissolution profile intralumenally. In contrast, the simulated plasma profile of atovaquone (Fig. 3B) is not affected by the different dissolution characteristics of this compound in media simulating the fasting gastric contents.

4. Discussion

To date, various media have been proposed for simulation of fasting gastric contents [1–5]. However, in these media, non-physiologically relevant surface active agents, lower than physiological pH values and/or high concentrations of physiological components were utilized. Sodium lauryl sulfate, the most frequently used artificial surfactant in dissolution testing, is known to hydrolyze in solutions having pH lower than 4 [29] leading to inconsistent medium composition. Also, sodium lauryl sulfate interacts with gelatin at pH < 5 making its use with gelatin capsule products problematic [30]. Finally, artificial surfactants can interfere with salt formation rates of weak bases and, thus, dissolution can be affected in an artefactual way [31].

In the present study, we proposed a new medium, FaSSGF, that, compared to previously proposed media, constitutes a more accurate simulation of fasting gastric contents. Data in FaSSGF proved to be important for the prediction of the intralumenal dissolution profile of a weak base, a compound that is primarily dissolved during gastric residence. In contrast, dissolution of atovaquone in the fasting stomach is not important for the assessment of the absorption profile presumably because dissolution at this location contributes little to the overall in vivo dissolution.

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