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Research Article

Solid Lipid Particles for Oral Delivery of Peptide and Protein Drugs III — the Effect of Fed State Conditions on the *In Vitro* Release and Degradation of Desmopressin

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Abstract. The effect of food intake on the release and degradation of peptide drugs from solid lipid particles is unknown and was therefore investigated *in vitro* using different fed state media in a lipolysis model. Desmopressin was used as a model peptide and incorporated into solid lipid particles consisting of trimyristin (TG14), tripalmitin (TG16), and tristearin (TG18), respectively. Fasted state and fed state media with varying phospholipid and bile salt concentrations, as well as fed state media with milk and oleic acid glycerides, respectively, were used as the release media. The presence of oleic acid glycerides accelerated the release of desmopressin significantly from all solid lipid particles both in the presence and absence of lipase. The presence of oleic acid glycerides also reduced the degradation rate of desmopressin, probably due to the interactions between the lipids and the protease or desmopressin. Addition of a medium chain triglyceride, trilaurin, in combination with drug-loaded lipid particles diminished the food effect on the TG18 particles, and trilaurin is therefore proposed to be a suitable excipient for reduction of the food effect. Overall, the present study shows that strategies to reduce food effect, such as adding trilaurin, for lipid particle formulations should be considered as drug release from such formulations might be influenced by the presence of food in the gastrointestinal tract.

KEY WORDS: fed state; lipid-based drug delivery systems; lipolysis; oral drug delivery; peptide drug.

INTRODUCTION

Increasing interest has been given to solid lipid particles for oral delivery of peptide and protein drugs due to observations of improved oral bioavailability in rats (1–4). The elevated oral bioavailability has been ascribed to the ability of the particles to protect the incorporated drug from degradation in the gastrointestinal tract (GIT) (2,5,6) and/or by facilitating increased permeation through the intestinal epithelium (7,8). Understanding the processes involved in the particle degradation, the release, and the subsequent absorption of peptide or proteins from such systems is important to assess their usefulness for oral delivery of peptide or protein drugs. Improved understanding of the processes will also increase the ability to make rational choices of excipients for the lipid-based formulations.

In recent studies, the authors described an *in vitro* lipolysis model capable of assessing the release and degradation of peptides and proteins from solid lipid particles (9,10). Desmopressin was chosen as a model peptide drug to enable future *in vivo* experiments, as it can be absorbed orally although to a limited extent. The presence of trilaurin (TG12) particles in the biorelevant medium was found to accelerate the release of desmopressin from solid lipid microparticles (SLM), as well as reduce the degradation of desmopressin by protease (10). Significant protection of desmopressin from proteolytic degradation has also been observed previously in the presence of a short chain (C6) monoglyceride (11).

To the best of our knowledge, studies on the influence of fed state conditions on solid lipid particle systems for oral delivery of peptide drugs have not previously been reported. The presence of food in the GIT could be troublesome because lipid-based drug delivery systems could be affected by food components and changes in the physiological conditions in the GIT. The bioavailability of desmopressin in conventional tablets has been shown to be affected by ingestion of food; the bioavailability of orally administered desmopressin was higher in the fasted state than after a standard meal in healthy adults (12). However, the food effect was not observed in children (13). After food ingestion, a wide array of triglycerides (TG) is expected to be present in the duodenum. Digestion of these TGs will result in a mixture of di- and

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monoglycerides, which can act as surfactants (14,15) and thereby influence the drug release process from the SLM. Therefore, studies are needed to investigate the influence of food components on the drug release from SLM.

As desmopressin is also used for treatment of central diabetes insipidus in infants (16), a medium based on milk would be appropriate to mimic that situation. Milk is a source of a variety of dietary lipids for *in vitro* systems due to the content of TG with a wide range of fatty acids, regarding both the chain length and the level of unsaturation (17). Milk has been used as a medium for dissolution studies (18–21) and in a lipolysis model (22) to simulate the fed gastric and intestinal environment. Multiple poorly soluble drugs were found to exhibit very different drug release behavior in milk compared to the fed state simulated intestinal medium (18,21). Although milk is suitable as a medium for lipolysis, the analysis of peptide degradation is hindered by the generation of a multitude of peptide fragments arising from the simultaneous degradation of milk proteins.

Oleic acid glycerides are very predominant in western diets, for instance, olive oil contains about 70% of oleic acid, while other vegetable oils (25–50%) as well as beef and poultry fat also contain appreciable levels (23). A mixture of mono-, di-, and triglycerides based on oleic acid can therefore be considered relevant for a medium simulating the fed state condition in the intestine (24), and media based on these glycerides will be less complex than a medium containing milk for drug release and degradation studies. This makes the analysis of peptide degradation feasible because the peptide drug will be the only substrate for proteases.

In the present study, the release of desmopressin from SLM was investigated in various media simulating fed state conditions in the duodenum. The differences between the drug release under fasted and fed state conditions are discussed based on results from SLM made using TGs with different fatty acyl groups, as well as the overall importance of fed state conditions on such systems for oral drug formulations. The degradation of desmopressin was also assessed in the different media to evaluate the drug degradation rate.

MATERIALS AND METHODS

Materials

The TGs Dynasan 112 (glyceryltrilaurate, TG12), Dynasan 114 (glyceryltrimyristate, TG14), Dynasan 116 (glyceryltripalmitate, TG16), and Dynasan 118 (glyceryltristearate, TG18) were kindly provided by Cremer Oleo (Hamburg, Germany). Peceol (Composition in Table I) was obtained as a gift from Gattefossé (Lyon, France). *Thermomyces lanuginosus* lipase A solution was a gift from Novozymes (Bagsværd, Denmark). Gum Arabic was obtained from Alfa Aesar (Karlsruhe, Germany). Phosphatidylcholine (Lipoid S PC, >98%) was purchased from Lipoid (Ludwigshafen, Germany) and desmopressin acetate (>98%) was obtained from Zhejiang Medicines & Health Products I/E (Chengguan Town, China). Sodium taurocholate (>95%), glycine (>99%), α-chymotrypsin from bovine pancreas (specific activity obtained from supplier, 65.6 U/mg against benzoyl-L-tyrosine ethyl ester), oleic acid (technical grade, >90%), glycerol (>99%), lauric acid (>99%), tributyrin (>99%), and polyvinyl alcohol (average M_w 85,000–124,000, 87–89% hydrolyzed) were acquired from Sigma-Aldrich (St. Louis, MO, USA). Whole fat (3.5%) milk (long life heat-treated and homogenized, DMK GmbH, Zeven, Germany) was purchased commercially.

Lipase Activity Determination

The activity of the lipase was tested against a substrate tributyrin based on a method obtained from the supplier. A gum arabic emulsifier was prepared by mixing NaCl (0.31 M), KH_2PO_4 (3.0 mM), gum Arabic (0.6% *w*/*v*), and glycerol (54% *v*/*v*) and the pH value was adjusted to 4.5. The emulsifier was mixed with tributyrin and water in the ratio 3:10:47 (*v*/*v*/*v*). This mixture was homogenized for 3 min using a T 18 ULTRA-TURRAX (IKA, Staufen, Germany) and kept for stirring at least 20 min at room temperature; thereafter, the pH value was adjusted to 4.8. This emulsion was used as the substrate for the lipase activity measurements. The experiments were conducted by adding 15 mL of substrate to a thermostated (37°C) vessel. The lipase solution was diluted to around 2 U/mL in 0.1 M glycine buffer (pH= 10.8); 1 mL of the diluted lipase solution was added to the vessel to initiate the lipolysis reaction. The pH was kept at 7.0 during the lipolysis by continuous addition of 0.1 M NaOH for 10 min; the added amount of NaOH during 5 min of linear addition was used to calculate the lipase activity. The amount of lipase used in the lipolysis studies was determined based on the quantified activity.

Preparation of SLM

SLM were prepared and loaded with desmopressin using the method described previously (10). In short, desmopressin in aqueous solution (50 mg/mL) was incorporated in lipid matrices consisting of TG14, TG16, and TG18, respectively. The SLM were produced using melt dispersion by emulsifying the desmopressin solution (100 μL) in the melted lipid (300 mg) and 3 mL of a 1% (*w*/*v*) polyvinyl alcohol (PVA) solution at 80°C. This emulsion was then whirlmixed and poured into a larger volume of cold (4°C) surfactant solution (150 mL, 0.1%, *w*/*v*, polyvinyl alcohol). The SLM were washed with 60 mL of Milli-Q water, filtered, and dried. The following day, the amount of desmopressin in the particles was determined by dissolving 10.0 mg particles in 2 mL of chloroform and extracting the desmopressin with 3 mL of Milli-Q water. The desmopressin content in the aqueous phase was determined by HPLC. The drug loading has been published earlier, as 0.77±0.08, 0.90±0.05, and 0.84 ±0.10 for the TG14, TG16, and TG18 particles, respectively (10).

Lipolysis Media

Three different fed state media were used to investigate their influence on the drug release (Table I). All the media were based on the FaSSIF-V2 and FeSSIF-V2 media described by Jantratid *et al*. (20), that are well characterized and based on data obtained from human duodenal aspirates. For the previously conducted fasted state studies (10),

Table I. The Composition of the Simulated Intestinal Media Used

	Fasted state	Fed state			
		No lipid	Glyceride	Milk	Oleic acid
Sodium taurocholate (mM)	3	10	10	10	10
Soy phosphatidylcholine (mM)	0.2	2	2	2	2
Maleic acid (mM)	19.1	55.0	55.0	–	55.0
Sodium chloride (mM)	68.6	125.5	125.5	–	125.5
Milk (mL)	–	–	–	20	–
Peceol[a] (mL)	–	–	0.77	–	–
Oleic acid (mL)	–	–	–	–	0.72
Lipase activity (U/mL)	571	1142	1142	1142	1142
Total volume (mL)	35	35	35	35	35

[a] Content from certificate of analysis 45.8% monoglyceride, 42.2% diglyceride, and 8.9% triglyceride (78.9% oleic acid, 12.2% linoleic acid)

FaSSIF-V2 was used directly. To investigate the effect of bile salt and phospholipid, the FeSSIF-V2 medium was also used without extra lipid components apart from the phospholipids. In the glyceride medium, the sodium oleate and monoolein in the FeSSIF-V2 medium were substituted with Peceol, which contains a mixture of mono- (45.8%), di- (42.2%), and triglycerides (8.9%) of oleic acid (78.9%) and linoleic acid (12.2%). This creates a more dynamic situation which would resemble the *in vivo* situation closer, where the lipase degrades the di- and triglycerides to fatty acids and monoglycerides. Studies have shown that the ratio between monoglycerides and diglycerides in the duodenum 60 min after administration of a high fat meal to the human stomach is around 1:1, similar to the peceol mixture (25). In the milk medium, the bile salt and phospholipid levels were kept the same as the other fed state media. As the milk mixture has a high buffer capacity and osmolality due to the varied milk components, no buffer or sodium chloride was added to this medium. A medium with oleic acid was also prepared (Table I), but only used for evaluating the release of desmopressin without lipase and degradation of desmopressin in the medium.

In Vitro Release Studies in a Lipolysis Model

Thirty-five milliliters of simulated fed state intestinal fluid was added to a thermostated glass vessel (37°C) and the medium was adjusted to pH 6.5 by addition of 1 M NaOH under stirring with a propeller stirrer. The SLM (100 mg) were added as free particles to the medium and allowed to disperse for 2 min, after which a sample was withdrawn (0 min sample). In the experiments with TG12, 200 mg of drug-free TG12 SLM was added together with the other drug-loaded SLM. *Thermomyces Lanuginosus* lipase A (571 U/mL or 1,142 U/mL) was added to initiate the lipolysis. Similar experiments were also performed without lipase. After lipase addition, 0.5 M $CaCl_2$ dosing was initiated from a dosing unit at a rate of 0.01 mL/min. During the following 120 min, the pH was maintained at 6.5 by automatic addition of 1 M NaOH. Samples of 500 μL were collected at 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, and 120 min and acidified with 50 μL of 1 M HCl to inhibit the lipase and protease activities. The samples were centrifuged for 3 min at 15,000 rpm (19,000×*g*) at ambient temperature; clear supernatants devoid of SLM were obtained. A sample of the supernatant was immediately analyzed by HPLC for determination of the concentration of desmopressin in the samples. After the lipolysis, the pH value of the medium was adjusted to 9.0 by addition of 1.0 M NaOH to ensure total ionization of the free fatty acids (FFA) generated. The NaOH addition occurring from the degradation of phospholipid in the medium was determined from an *in vitro* lipolysis experiment without SLM (blank).

The amount of FA hydrolyzed at various time points was calculated based on the amount of NaOH added during the lipolysis experiments. Values from the blank experiments without SLM were subtracted as background. The total amount of FA hydrolyzed after complete lipolysis was calculated using the volume of NaOH added after adjusting to pH 9.

Release of Desmopressin in the Presence of Fatty Acid

Fasted state medium (5 mL) with either 3.1 mM of oleic acid, 3.1 mM of lauric acid, or no fatty acids was rotated for 60 min with 10.0 mg of TG18 SLM. A sample was collected and analyzed similarly to the studies of desmopressin release.

In Vitro Degradation of Desmopressin

Degradation experiments were conducted in a similar setup as the release studies but with α-chymotrypsin as protease. The protease was chosen as it readily degrades desmopressin, while other proteases like trypsin and pepsin do not (26,27). Nonencapsulated desmopressin (1.0 mg) was added to the medium being tested and at time 0 min, α-chymotrypsin (10.0 mg, 18.7 U/mL) was added. Samples were collected at 0, 15, 30, 45, and 60 min and treated and analyzed similarly to samples from the release studies.

To test the stability of desmopressin during formulation of SLM, a desmopressin solution (50 mg/mL) was kept at 80°C for 30 s and then rapidly cooled to 5°C. Samples were withdrawn before and after the heating/cooling treatment, and the concentration of desmopressin was determined by HPLC. After the treatment, 94.6±3.2% of the desmopressin was still intact, and desmopressin was therefore considered to be stable during the preparation of SLM.

Analysis of Desmopressin by HPLC

For quantitative determination of desmopressin, samples were analyzed using a Dionex HPLC system equipped with a P680 HPLC pump, an ASI-100 automated sample injector, a PDA-100 photodiode array detector detecting desmopressin at a wavelength of 220 nm, and a C18 column (4.60×100 mm, 5 μm) from Phenomenex (Torrance, CA, USA) was employed. A binary solvent system was used at room temperature at a flow rate of 1 mL/min. Solvent A was 0.1% (*v*/*v*) trifluoroacetic acid in Milli-Q water and solvent B was acetonitrile. A gradient system running for 18 min was used; 0–2 min 23% B, 2–4 min 23–100% B, 4–14 min 100% B, 14–16 min 100–23% B, and 16–18 min 23% B. Desmopressin had a retention time of 2.6 min and was quantified using a calibration curve in the linear range of 5–100 μg/mL (R^2=0.999) having an y-axis intercept of 0.144 and a limit of detection of 0.1 μg/mL. Inter-day variation was tested over 2 days, and relative standard deviations of 2.0–9.2% were found over the different standard concentrations.

Theoretical Calculations

Area under the curve (AUC) values were calculated using the trapezoidal rule on the data from the release curves.

The following equation (Eq. 1), derived from first order degradation kinetics, was used to calculate the theoretical release of desmopressin in the presence of protease. The validity of the equation was tested by performing lipolysis experiments on TG16 SLM in the different media with 18.7 U/mL of α-chymotrypsin.

$$Q_{calc,t} = \left(Q_{\triangle t} + Q_{calc,t-1}\right) * e^{-k*\triangle t} \quad (1)$$

Where $Q_{\mathrm{calc},t}$ is the calculated amount of desmopressin released at time point t (min), $Q_{\triangle t}$ is the amount of desmopressin released between the current and the last time point during the lipolysis experiments (in the presence of lipase), $Q_{\mathrm{calc},t-1}$ is the amount of desmopressin calculated to be present at the last time point, k (min^{-1}) is the degradation constant for desmopressin in the lipolysis medium containing α-chymotrypsin, and $\triangle t$ is the time between the current and last time point (min).

Statistics

All data are presented as mean±SD of triplicates unless otherwise stated. Statistical analysis was performed using GraphPad Prism software version 6.0 (GraphPad Software, CA, USA). One-way ANOVA was used and multiple comparisons were done using Newman-Keuls test.

RESULTS AND DISCUSSION

The Presence of Oleic Acid Glycerides Accelerates the Release of Desmopressin

Increased initial burst release of desmopressin (at time zero, after 2 min of dispersion but before lipase addition) was observed in the media containing lipids (milk and glyceride media, Fig. 1). The fasted and fed state media without lipids resulted in a desmopressin burst release of 4.4±0.9% and 8.0 ±3.2% at 0 min, respectively, while the milk medium and the glyceride medium resulted in a burst release of 20.0±5.7% and 34.3±9.3%, respectively (n=6, lipase was added immediately after the sample, therefore both data from experiments in the presence and absence of lipase used). Proteins in the milk and partial glycerides in the glyceride medium are surfactants, which can interact with the SLM and cause the increased burst release of desmopressin. In the absence of lipase (Fig. 1a), the increased amounts of surfactants in the fed state (no lipid) medium disrupted the TG14 SLM and thereby increased the release of desmopressin compared to the fasted state medium. In the milk medium, minimal desmopressin release from the TG14 SLM was observed in the absence of lipase, while in the fed state glyceride medium, the TG14 SLM released the desmopressin content very quickly (Fig. 1a). The glyceride mixture contains a lot of monoglycerides, which can influence drug release from SLM due to their surfactant capabilities, by solubilizing lipids and transforming them into mixed surfactant-lipid micelles (28).

The release of desmopressin in the presence of lipase (Fig. 1b) was similar in fasted and fed state (no lipid) media although the fed state conditions created a small lag time in the desmopressin release. The release of desmopressin in the milk medium in the presence of lipase was delayed compared to the media without lipids. The retarded drug release might be caused by substrate competition between the lipids present

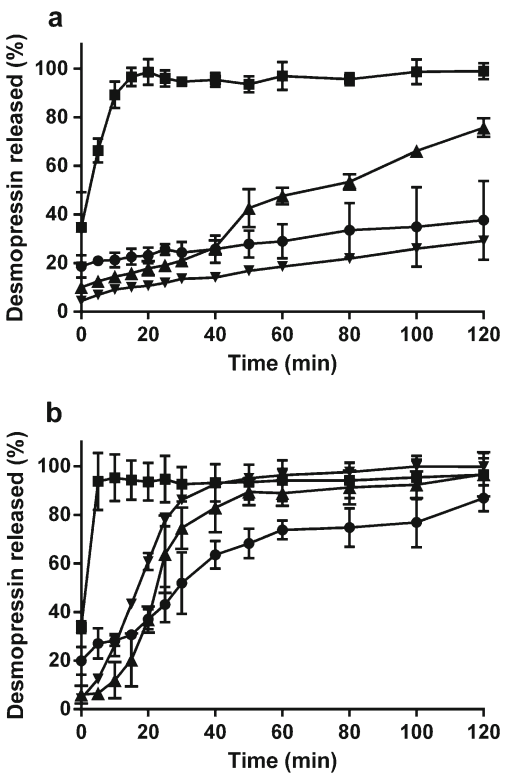

Fig. 1. Release of desmopressin from TG14 SLM in the absence **a** and presence **b** of lipase. *Filled square* fed state (glyceride), *filled circle* fed state (milk), *filled triangle* fed state (no lipid), *inverted filled triangle* fasted state. Fasted state data were published in a previous publication (10)

in the milk and the TG14 SLM. In the glyceride medium, the release of desmopressin from the TG14 SLM was very fast in the presence of lipase, similar to what was observed without lipase. The SLM were finely dispersed in the glyceride medium compared to the other media, suggesting increased wetting of the SLM by the high content of monoglycerides in this medium, which could be a major factor influencing the desmopressin release rate. The results suggest that administration of TG14 SLM after ingestion of food rich in fat might significantly increase the release of drug in the GIT.

The glyceride medium also increased the burst drug release from the TG16 SLM ($p<0.05$, $n=6$, Fig. 2) but not from the TG18 SLM (Fig. 3) compared to the lipid free and milk media. The release of desmopressin from TG16 was similar in the fasted and fed state (no lipid) medium after 120 min of lipolysis without lipase ($p>0.05$, Fig. 2a). For the TG18 SLM, almost no drug release was observed without lipase in those media (Fig. 3a). This was contrary to the TG14 SLM, where the release of desmopressin was faster in the fed state (no lipid) medium compared to the fasted state medium in the absence of lipase. These results suggest that the increased bile salt and phospholipid levels are not sufficient to increase the release of desmopressin from the slightly more lipophilic TG16 and TG18 SLM. This would mean that even though the ingestion of triglyceride particles might trigger increased release of bile salts and phospholipid into the intestine, this would not be expected to alter the drug release from these particles. In the absence of lipase, there was almost no release of desmopressin from TG16 and TG18

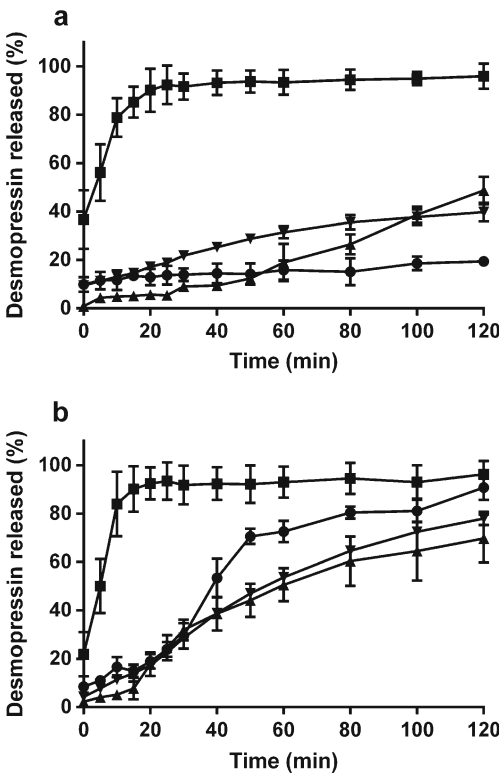

Fig. 2. Release of desmopressin from TG16 SLM in the absence **a** and presence **b** of lipase. *Filled square* fed state (glyceride), *filled circle* fed state (milk), *filled triangle* fed state (no lipid), *inverted filled triangle* fasted state. Fasted state data were published in a previous publication (10)

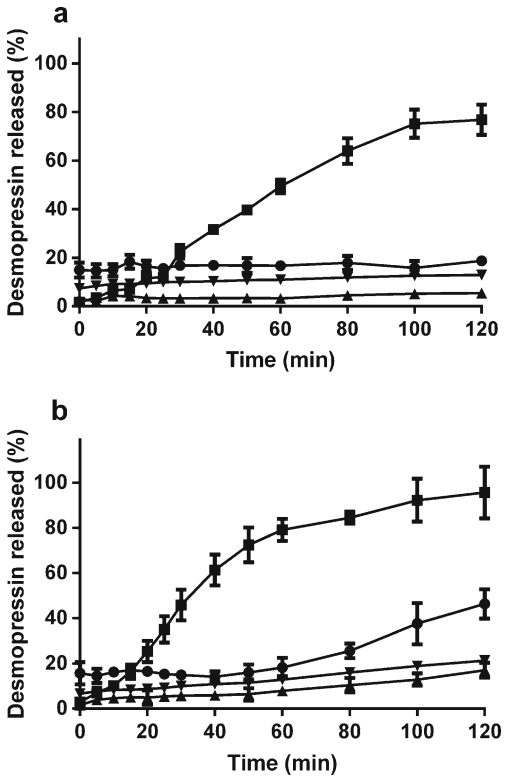

Fig. 3. Release of desmopressin from TG18 SLM in the absence **a** and presence **b** of lipase. *Filled square* fed state (glyceride), *filled circle* fed state (milk), *filled triangle* fed state (no lipid), *inverted filled triangle* fasted state. Fasted state data were published in a previous publication (10)

SLM in the milk medium. The drug release in the milk medium was found to be even slower than that in the lipid free media for the TG16 SLM (Fig. 2a). The TG16 SLM behaved similarly to the TG14 SLM in the glyceride medium, where a fast release of desmopressin was observed in the absence of lipase (Fig. 2a). The release of desmopressin from TG18 SLM in the glyceride medium was also accelerated (Fig. 3a), 76.8±6.2% of the encapsulated desmopressin were released after 120 min. As no lipolysis occurs in this case, the desmopressin release must be primarily attributed to the interaction of SLM with the glycerides present in the medium facilitating diffusion of desmopressin from the particles.

In the presence of lipase, the release of desmopressin in the fasted and fed state (no lipid) media was similar after 120 min of lipolysis ($p>0.05$) for both TG16 and TG18 SLM (Figs. 2b and 3b). This indicates that the increased bile salt and phospholipid content in the fed state medium had no influence on the lipolytic degradation of these SLM, similar to what was observed for the TG14 SLM. The release of desmopressin from the TG16 and TG18 SLM in the milk medium with lipase (Figs. 2b and 3b) was increased compared to the lipid free media, indicating that the digestion products of milk lipids influence the TG16 and TG18 SLM to a larger extent than the TG14 SLM (Figs. 1b, 2b, and 3b). This can be explained by the increased demand of surfactants to facilitate digestion of the very insoluble long chain TG as well as drug release from these particles. Even though increased levels of bile salt and phospholipid have higher acceleration of lipolytic

degradation for long chain TG than medium chain TG (29), it did not increase the release of desmopressin from the SLM, whereas the presence of lipid digestion products did. This underlines the importance of the lipid digestion in the process of drug release from solid lipid particles. Similarly as for the TG14 SLM, a fast release of desmopressin from TG16 and TG18 particles was observed in the glycerides medium in the presence of lipase (Figs. 2b and 3b). Possible explanations for the accelerated drug release could be (i) interactions between the solubilized glyceride molecules and the SLM leading to destabilization of the particles, (ii) interactions between the SLM and glyceride emulsion droplets possibly solubilizing the lipid, and/or (iii) facilitation of particle lipolysis caused by improved wetting of the particles making more surface area available for the lipase to act upon.

To determine if oleic acid could have a role in the accelerated release of desmopressin from SLM, TG18 SLM were incubated for 60 min in fasted state medium with oleic acid (3.1 mM) and the release of desmopressin was compared to that in the fasted state medium. The presence of oleic acid in the medium was found to have a significant effect on the desmopressin release, 95.2±5.7% of desmopressin was released from TG18 SLM in the presence of oleic acid compared to 12.8±2.1% in fasted state medium ($p<0.001$). Fatty acids are known to induce transformation of liquid crystalline phases, which cause significant changes of their functional properties due to assembly in different symmetries (28). This could affect the release of a drug compound from the particles. In future studies, it would be interesting to investigate if the interaction between the SLM and oleic acid is solely due to surfactant effects or if other mechanisms are predominant. Conclusively, the presence of oleic acid

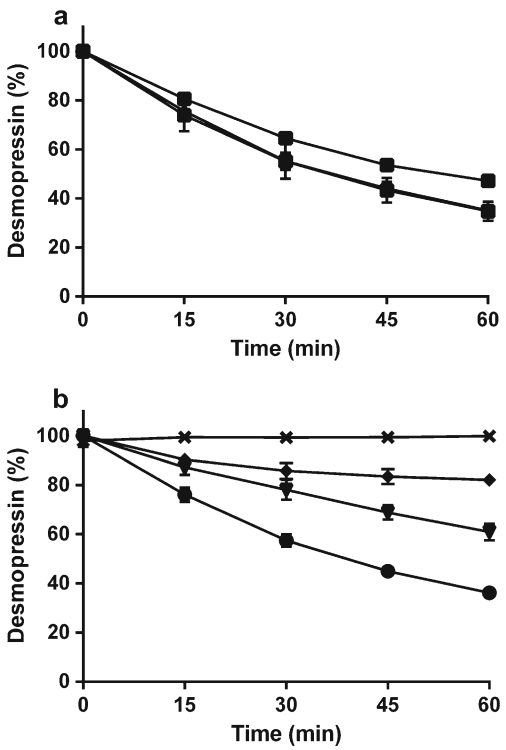

Fig. 4. Degradation of desmopressin by α-chymotrypsin in **a** media without lipid and **b** media with lipid in the presence of lipase unless otherwise specified. *White square* and *filled triangle* plots are superimposed. *Filled square* fasted state (no calcium), *white square* fasted state, *filled triangle* fed state (no lipid), ***X*** fed state (oleic acid, no calcium), *black diamond suit* fed state (glyceride, no calcium), *inverted filled triangle* fed state (glyceride), *filled circle* fed state (glyceride, no lipase)

Table II. Apparent First-Order Degradation Constants Calculated from the Degradation Studies and Used for the Calculation of Desmopressin Release Profiles

	Degradation constant (min^{-1})	r^2
Fasted state	0.0187±0.00	0.992±0.01
Fed state (no lipid)	0.0186±0.00	0.996±0.01
Fed state (glyceride)	0.0092±0.00	0.999±0.00

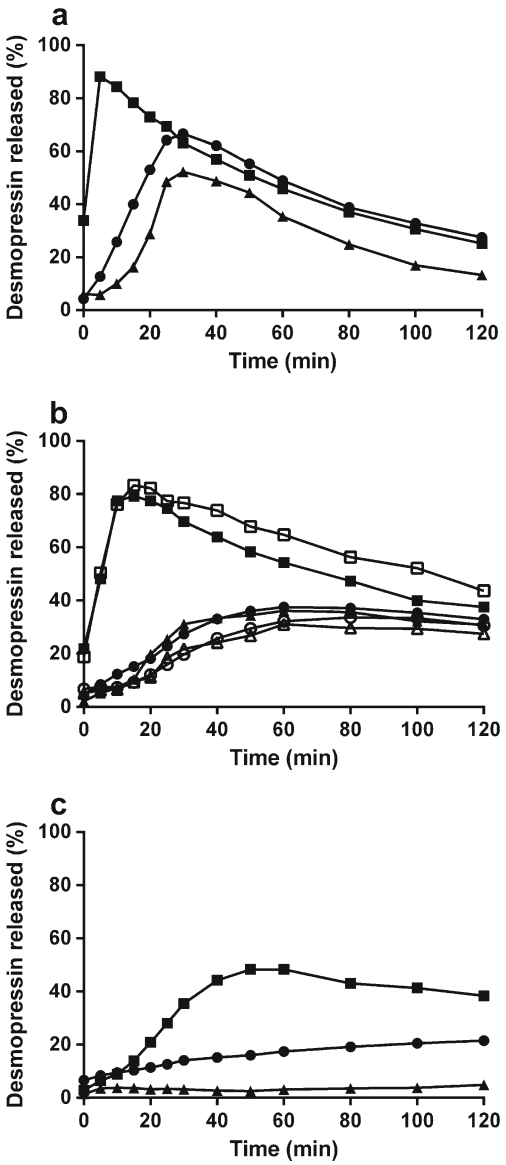

Fig. 5. Calculated desmopressin release profiles from SLM in the presence of α-chymotrypsin over 120 min. **a** TG14 SLM, **b** TG16 SLM, and **c** TG18 SLM. In **b**, open symbols are measured lipolysis data. *Filled square* fed state (glycerides), *filled circle* fasted state, *filled triangle* fed state (no lipid)

glycerides stimulated the release of desmopressin from all SLM similar as oleic acid, so oleic acid seems to be a major factor for the accelerated drug release.

Oleic Acid Inhibits Desmopressin Degradation

The degradation of desmopressin by α-chymotrypsin was investigated in the different media (Fig. 4), except the milk medium as the presence of degraded milk proteins hindered analysis of desmopressin. Incubation of nonencapsulated desmopressin in the fasted and fed state (no lipid), and fed state (glyceride, but no lipase) resulted in similar desmopressin degradation at 60 min (approximately 65%, Fig. 4a, b). In the presence of lipase, less desmopressin was degraded in the fed state glyceride medium, suggesting a protease-inhibiting effect of the lipolysis products either by interaction with the protease or with the substrate. Similar inhibition effect for protease has been reported previously with trilaurin lipolysis products as well as a short chain monoglyceride (10,11). The inhibition effect was more profound when no calcium was added to the medium (Fig. 4b) probably due to impairment of the effect by precipitation of generated fatty acids with calcium. To clarify if the oleic acid is a protease-inhibiting compound, pure oleic acid was added in an amount corresponding to full degradation of the glycerides in the fed state glyceride medium. Complete inhibition of the proteolytic activity was observed (Fig. 4b), suggesting that oleic acid can effectively inhibit the degradation of desmopressin. The presence of calcium in the medium also affected the desmopressin degradation; the peptide was degraded slower in the fasted state medium without calcium (52.9±0.3% degraded) than in the medium with calcium (65.2±3.9% degraded, $p<0.05$, Fig. 4a). This could also account for some of the inhibition observed from the lipolysis products, as the removal of calcium from the medium by precipitation with fatty acids might slow down the proteolytic reaction. Reduced degradation of desmopressin in the fed state would be expected to provide higher bioavailability than in the fasted state. The opposite has been observed in an *in vivo* study in healthy adults where the bioavailability of desmopressin was reduced by administering it 1.5 h after food intake (12). An explanation could be that the reduced degradation of desmopressin is counteracted by the higher concentration of proteases, which are released from the pancreas together with other enzymes in the fed state.

TG12 Diminishes the Food Effect

The lipolytic degradation products of TG14, TG16, and TG18 SLM have previously been shown to have no effect on the degradation of desmopressin by α-chymotrypsin. Moreover, the release of desmopressin from SLM in the presence of protease could be predicted from measured release data in the absence of protease and the degradation constant from the degradation of nonencapsulated desmopressin (10). Therefore, the desmopressin degradation constants calculated from the degradation studies in the different media (Table II) were used to predict the differences in the release of desmopressin from the SLM in the various biorelevant media in the presence of protease (Fig. 5). The validity of these calculations was proven through experiments using the TG16 SLM. It was observed that the calculated release profiles did resemble the measured release data for desmopressin (Fig. 5b). Therefore, the calculation can be used to predict the release profile for desmopressin undergoing simultaneous degradation by α-chymotrypsin.

As expected from the measured desmopressin release data (Figs. 1b, 2b and 3b), the release of desmopressin in the glyceride medium resulted in higher amounts of desmopressin in the medium during the 120 min lipolysis study due to the reduced drug degradation as well as the faster drug release in comparison to the other media. The area under the curve (AUC) values for the theoretic drug release profiles were calculated (Table III) to quantify the availability of desmopressin in the intestine during the 120 min. Based on these calculations, less desmopressin would be available for absorption in the fed state (no lipid) medium for all SLM compared to the fasted state. The AUC values for the glyceride medium were much higher than for the other media as expected based on the drug release curves ($p<0.05$, Table III). The ratio between the AUC in the glyceride medium and in the fasted state medium indicates to which degree the particles are affected by the fed state conditions. While all SLM were significantly affected by the fed state glyceride conditions ($p<0.05$, Table III), the SLM with longer chain TG18 were affected more profoundly. A possible strategy for minimizing the food effect on SLM is to dose the SLM with easily digestible drug-free TG12 SLM. The fed/fasted AUC ratio decreased from 7.0 to 1.4 for TG18 SLM with the addition of TG12 SLM (TG18:TG12 1:2 *w*/*w*, Table III). These results suggest that administration of TG12 SLM with these lipid particle formulations is likely to diminish the food effect by releasing lipid digestion products capable of accelerating drug release from the particles.

To investigate if lauric acid had a similar effect on the desmopressin release as oleic acid, TG18 SLM were incubated in the fasted state medium with lauric acid (3.1 mM). Lauric acid was found to be a slightly less potent drug release enhancer than oleic acid, 84.0±8.1% and 95.2±5.7% of desmopressin was released, respectively, after 60 min incubation in the presence of lauric acid and oleic acid, but the difference was not statistically significant ($p>0.05$). These results suggest that trilaurin diminishes the food effect by releasing lauric acid, which has a similar effect on the release

Table III. The AUC Values (%×Min) for the Calculated Desmopressin Release Profiles in the Presence of α-Chymotrypsin as well as the Ratio Between AUC in the Fed State (Glyceride) Medium and the Fasted State Medium

SLM	Fasted state	Fed state (no lipid)	Fed state (glyceride)	Fed state (glyceride)/fasted state ratio
TG14	3,810	3,388	6,063	1.6
TG16	2,301	2,107	5,714	2.5
TG18	627	411	4,384	7.0
TG18+TG12 (1:2)	6,759	–	9,198	1.4

AUC area under the curve, *SLM* solid lipid microparticle, *TG12* trilaurin, *TG14* trimyristin, TG16 tripalmitin, *TG18* tristearin

of desmopressin as oleic acid. It needs to be established in future studies if the accelerated drug release is caused by a general fatty acid—triglyceride interaction. The accelerated release is assumed to have clinical relevance as the fatty acid levels after a high fat meal were found to be much higher (52 mM, 30 min after administration) than what was tested in these experiments, and even 210 min after administration of the meal, the solubilized amount of fatty acids were found to be 5.2 mM (25). This could potentially have huge implications for the behavior of triglyceride particles in the GIT.

It should be noted that the model established in this study only simulates the duodenum. The stomach would also play an important role for the success of a solid lipid particle formulation. After ingestion of fat-rich meals, the gastric emptying is decreased which will influence the residence time of the formulation in the stomach (30). Additionally, the presence of gastric lipase, pepsin, and acidic pH could trigger drug release from the solid lipid particles. A model simulating the conditions in the stomach could therefore be established and possibly combined with the duodenal model to obtain a better prediction of the processes in the GIT.

The present study shows that food effect should be considered when dosing solid lipid particles. The presence of food in the intestine is expected to severely affect the release of drug from solid lipid particles, as well as the degradation of the drug compound in the case of peptides. Therefore, *in vivo* studies addressing both the fasted and the fed state should be conducted to investigate the relevance of the observed effects in an *in vivo* setting. Although the dosing of drug-loaded SLM might lead to a higher drug bioavailability *in vivo* in the fed state due to the accelerated drug release and inhibited enzymatic degradation, large variations are expected dependent on the composition of the food ingested, as the release profile for desmopressin was very different in the milk medium compared to the fed state glyceride medium. This would especially be apparent in different patient groups, as infants being fed primarily milk would show a different drug release profile than adults being fed western style diets containing a high content of oleic acid glycerides.

CONCLUSION

Solid lipid particles containing the model peptide desmopressin showed accelerated drug release in the medium containing oleic acid glycerides simulating fed state conditions in the GIT. The same medium also decreased the degradation of desmopressin by α-chymotrypsin, especially oleic acid was found to protect desmopressin significantly. Addition of trilaurin together with the solid lipid formulations diminished the observed *in vitro* food effect on drug release, which could make it a useful excipient for reducing food effect. Based on these *in vitro* findings, considerations on the effect of fed state conditions should be taken when testing solid lipid particles for oral administration.

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