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RESEARCH PAPER

Solid Lipid Particles for Oral Delivery of Peptide and Protein Drugs II – The Digestion of Trilaurin Protects Desmopressin from Proteolytic Degradation

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ABSTRACT

Purpose To investigate the *in vitro* release and degradation of desmopressin from saturated triglyceride microparticles under both lipolytic and proteolytic conditions.

Methods The release of desmopressin from different solid lipid microparticles in the absence and presence of a microbial lipase and protease was determined. Trilaurin (TG12), trimyristin (TG14), tripalmitin (TG16), and tristearin (TG18) were used as lipid excipients to produce solid lipid microparticles.

Results In the presence of lipase, the rate of drug release from different lipid particles was in the order of TG14 > TG16 > TG18, which is the same rank order as the lipid degradation rate. A reverse rank order was found for the protection of desmopressin from enzymatic degradation due to spatial separation of desmopressin from the protease. TG12 accelerated the release of desmopressin from all lipid particles when added as either drug-free microparticles to the lipolysis medium or incorporated in TG16 particles. Additionally, TG12 particles protected desmopressin from degradation when present in the lipolysis medium with the other lipid microparticles.

Conclusions TG12 is a very interesting lipid for oral lipid formulations containing peptides and proteins as it alters release and degradation of the incorporated desmopressin. The present study demonstrates the possibility of bio-relevant *in vitro* evaluation of lipid-based solid particles.

KEY WORDS lipid hydrolysis · peptide and protein drugs · proteolysis · solid lipid particles · triglycerides

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INTRODUCTION

Solid lipid particles are promising carriers for oral delivery of peptide and protein drugs. Reports have shown that such carriers protect the macromolecules from degradation in the gastrointestinal tract (GIT) (1–3) and possibly enhance the membrane permeation of the macromolecules (4,5). This was evidenced by enhanced bioavailability of peptides like insulin and salmon calcitonin in rats *via* oral administration (1,6–8).

To obtain information about the efficacy of lipid systems when taken orally, it is important to determine the release characteristics of an encapsulated drug when subjected to conditions simulating the environment in the GIT. In a recent study, an *in vitro* lipolysis model was described for investigating the release of proteins from solid lipid microparticles (SLM) (9). The study showed that triglyceride (TG) formulations exhibit a lipase-mediated release of lysozyme, and that the length of the triglyceride fatty acid chains affected the rate of drug release. The method provided valuable information about the release mechanism of lysozyme from SLM during lipolysis in the medium simulating the intestinal fluids. However, the degradation of peptides and proteins by proteases is also an important factor for the amount of drug available for absorption (10,11). The sustained release capabilities of solid lipid particles might protect peptides due to spatial separation of peptide from protease, but a prolonged release of peptide can also result in incomplete release in the GIT.

In the present study, desmopressin was used as a small model peptide drug and incorporated into SLM made of different saturated TG, and the release and degradation of desmopressin from SLM were investigated. A peptide-degradation step using α-chymotrypsin as protease was added in the previously established *in vitro* lipolysis model (9) because desmopressin is relatively stable in the presence of trypsin and pepsin, but it is readily degraded by α-chymotrypsin (12,13). The aim of this study was thus to allow assessment of the

dynamic process of peptide release caused by lipolysis of the lipid matrix and degradation of the peptide by protease. The model was then used to investigate the influence of lipid excipients on the simultaneous release and degradation of desmopressin from SLM.

MATERIALS AND METHODS

Materials

The TGs Dynasan 112 (trilaurin, TG12), Dynasan 114 (trimyristin, TG14), Dynasan 116 (tripalmitin, TG16) and Dynasan 118 (tristearin, TG18) were kindly provided by Cremer Oleo (Hamburg, Germany). *Thermomyces lanuginosus* lipase A solution (100,000 U/g) was obtained as a gift from Novozymes (Bagsværd, Denmark). Phosphatidyl choline (Lipoid S PC) was purchased from Lipoid (Ludwigshafen, Germany). Desmopressin (>98%) was purchased from Zhejiang Medicines & Health Products I/E (Hangzhou, Zhejiang, China). Sodium taurocholate (>95%), Tween 80, and α-chymotrypsin from bovine pancreas (65.6 benzoyl-L-tyrosine ethyl ester U/mg) were bought from Sigma-Aldrich (St. Louis, MO).

HPLC Analysis

For quantitative determination of desmopressin, samples were analyzed using a Dionex HPLC system equipped with a P680 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). A binary solvent system was used as the mobile phase at a flow rate of 1 mL/min at room temperature. Solvent A was 0.1% (v/v) trifluoroacetic acid (TFA) in Milli-Q water and solvent B was acetonitrile. A gradient system running for 18 min was used to ensure rinsing of lipids from the column; 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 standard calibration curve in the linear range of 5–100 μg/mL.

Preparation of Solid Lipid Microparticles

Microparticles were produced using the method reported previously (9), which was based on the method described by Reithmeier *et al.* (14). In short, an aqueous solution of desmopressin (50 mg/mL) was mixed with a lipid melt containing either a single lipid excipient or a mixture of TG12 and TG16 (20, 50 or 80% of TG12) at 80°C. Hot PVA solution (3 mL, 1% PVA, 80°C) was added and the mixture was whirlmixed. The mixture was then poured into 150 mL of cold (4°C) PVA solution (0.1% PVA). The obtained particles were filtered and dried overnight under vacuum. For the production of drug-free TG12 microparticles, Milli-Q water was added instead of the desmopressin solution. Three batches of each particle type were used for determination of particle size and drug loading (DL). For lipolysis studies, multiple batches were pooled.

Microparticle Characterization

The average diameter of the SLM was measured using laser diffraction method with a Mastersizer 2000 (Malvern, Worcestershire, UK). The samples were dispersed using a dry powder dispersion unit (Scirocco, Malvern, UK) at a feed pressure of 2.5 bar and analyzed in triplicate with a reference refractive index of 1.553. SPAN values were defined as the difference between the 10% and 90% quantile divided by the 50% quantile. The DL was determined by dissolving 10.0 mg of the SLM in 2 mL of chloroform, followed by extraction of desmopressin with 3 mL of Milli-Q water. The mixture was mixed for 15 min, and after phase separation, a 500 μL sample was taken from the aqueous phase and analyzed by HPLC using the method described above. Visualization of the surface morphology of the SLM was performed using a scanning electron microscope (JMS-5200, JEOL, Tokyo, Japan) with an accelerating voltage of 20 or 25 kV. Prior to the evaluation, the particles were coated with gold using an Auto Sputter Coater, E5200 (Bio-Rad Laboratories, Hercules, CA) using compressed argon at 1 bar. The amount of desmopressin on the surface of the particles was determined by incubating 15.0 mg of TG16 particles in 5 mL of a 5% Tween 80 solution for 30 min under continuous rotation. The amount of desmopressin in the solution was determined by HPLC after centrifugation for 3 min at 15000 rpm (19000 g at r_{max}).

Lipolysis

Sodium taurodeoxycholate is commonly used in lipolysis studies, it provides similar lipolysis rates as crude bile salt mixtures when emulsions containing long-chain lipids are digested *in vitro* (15,16). However, sodium taurocholate was selected in our studies because of the incompatibilities of sodium taurodeoxycholate with lysozyme, another model protein in our studies. The lipolysis medium (35 mL, Table I) was added to a thermo-stated glass vessel (37°C) and adjusted to pH 6.5 by addition of 0.125 M NaOH. SLM (100 mg) were added to the medium and 2 min after dispersion, a zero sample was withdrawn. In the experiments investigating the effect of drug-free TG12 microparticles, 10 mg or 100 mg of drug-free TG12 microparticles were added to the vessel together with the drug loaded particles.

Table I Composition of the Lipolysis Medium (29)

Component	Concentration (mM)
Sodium taurocholate	3.0
Phosphatidylcholine	0.2
Maleic acid	19.1
Sodium chloride	68.6

The lipase (571 U/mL) and/or α-chymotrypsin (18.7 U/mL) was added in the experiments where lipase and/or protease was needed. Experiments without enzymes were also conducted, to investigate the release of desmopressin in the medium. After adding the enzymes, addition of $CaCl_2$ (0.5 M) was initiated at a rate of 0.01 mL/min. During the following 120 min the pH was maintained at 6.5 by automatic addition of NaOH (0.125 M). Samples (500 μL) were collected at 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, and 120 min. In the presence of protease, the samples were immediately acidified with 20 μL HCl (1 M) to inhibit the activity of α-chymotrypsin (17). The samples were immediately centrifuged for 3 min at 15,000 rpm (19000 g at r_{max}) at room temperature to separate lipid particles from the medium. A sample of the supernatant (100 μL) was immediately transferred to a HPLC vial and the concentration of desmopressin was determined by HPLC. After the lipolysis experiment, the pH value of the medium was adjusted to 9.0 by addition of 0.125 M NaOH to ensure total ionization of the free fatty acids (FFA) generated. The background NaOH addition was determined from a lipolysis experiment without SLM. To determine the protease-mediated degradation of non-encapsulated desmopressin, 0.8 mg of desmopressin was added corresponding to the amount of peptide encapsulated in 100 mg particles. All experiments were run in triplicate.

The amount of hydrolyzable FA was calculated based on the composition of lipid excipients found in the certificate of analysis from the supplier, and the amount of FA hydrolyzed at various time points was calculated based on the amount of added NaOH. The total amount of FA hydrolyzed after 120 min of lipolysis was calculated using the amount of NaOH added after adjusting the pH of the medium to 9.0.

Calculations

DL was calculated using the following equation:

$$DL(\%) = \left(m_{drug}/m_{particles}\right) * 100 \quad (1)$$

where $m_{particles}$ and m_{drug} are the exact mass of the weighed particles and the total mass of the drug found in the weighed particles by HPLC analysis, respectively.

Apparent first-order degradation has been observed in multiple cases for protein degradation by proteases (18). This was also the case in these experiments, as the proteolytic degradation of desmopressin was found to be near-linear ($r^2 = 0.9535$) on a logarithmic scale. The following equation, derived from first order degradation kinetics, was therefore used to calculate the theoretical release of desmopressin in the presence of protease.

$$Q_{calc,t} = \left(Q_{\triangle t} + Q_{calc,t-1}\right) * e^{-k*\triangle t} \quad (2)$$

where $Q_{calc,t}$ 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), $Q_{calc,t-1}$ 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, $\triangle t$ is the time between the current and last time point (min)

The degradation was expressed as a degradation value (D) and calculated using the following equation:

$$D = \left(Degraded_{SLM}/Degraded_{desmopressin}\right) * 100\ \% \quad (3)$$

where $degraded_{SLM}$ and $degraded_{desmopressin}$ are the percentages of desmopressin degraded after 120 min of lipolysis in the presence of protease on SLM and non-encapsulated desmopressin, respectively.

The amount of desmopressin that could be available for absorption during the release experiments was calculated using the AUC of the release profiles:

$$Available = \left(AUC_{SLM}/AUC_{desmopressin}\right) * 100\ \% \quad (4)$$

where AUC_{SLM} and $AUC_{desmopressin}$ are the AUC of the release profiles during 120 min of lipolysis in the presence of protease on SLM and non-encapsulated desmopressin, respectively.

Statistics

The statistical analysis was performed using GraphPad Prism software version 5.0 (GraphPad Software, La Jolla, CA). One-way ANOVA was used and multiple comparison was done using Newman-Keuls test.

RESULTS AND DISCUSSION

Particle Characterization

All produced SLM were characterized with regard to drug load, particle size, and morphology (Table II and Fig. 1). Similar drug loading ($p>0.05$) was found for all the particles, except for the TG12 microparticles, which contained less than 0.1% of desmopressin and was therefore not included in the present study. All of the produced particles were similar in size except the TG18 microparticles, which were significantly smaller than the blend particles with 50 and 80% TG12 ($p<0.05$) and all particles had SPAN values below 2. The production of lipid blend particles containing TG12 and TG16 seemed to result in more variable batches as relative standard deviations on size measurements (19.74–23.76%) were larger for those particles compared to the single triglyceride particles (0.63–3.44%).

All particles were found to be spherical in shape and the particles made from a single TG possessed similar morphology (Fig. 1). Particle sizes observed on SEM-micrographs was in agreement with the measurements by laser diffraction. The particles composed of a mixture of TG12 and TG16 had different surface morphology. An increase in the amounts of TG12 in the particles resulted in less smooth particle surfaces. This might be caused by surface erosion of TG12 as a result of exposure to the surfactant solution during preparation of the particles (19). Similarly, alteration of the lipid crystal morphology due to the different polymorphic behavior of triglyceride mixtures could also cause surface modifications (20,21). These processes could contribute to the larger variation in the size of the produced particles and cause the inability to incorporate desmopressin into pure TG12 particles.

To evaluate the presence of desmopressin on the exterior of the particles, TG16 particles were incubated in a strong surfactant solution (5% Tween 80). This would release desmopressin adsorbed to the particle surface due to competitive adsorption processes between Tween 80 and desmopressin. After 30 min of incubation, 11.8±0.5% of desmopressin was released from the particles which indicate that the majority of the desmopressin is present in the particle interior.

Triglyceride Particles can Provide Sustained Release of Desmopressin Based on Lipid Chain Length

The release of desmopressin from TG microparticles was assessed using the *in vitro* lipolysis model in the absence of enzymes. It was found that 29.8±6.4%, 39.7±3.8% and 12.9±1.2% of desmopressin was released from the TG14, TG16 and TG18 microparticles after 120 min, respectively. The difference in the release of desmopressin from TG14 and TG16 microparticles was not statistically significant ($p>0.5$).

Table II Drug Loading (DL) and Size Data for the Produced Particles

Formulation (w/w)	DL (%)	D50 (μm)	SPAN
TG14	0.77±0.08	34.9±1.2	$1.72\pm0.12^{a,b}$
TG16	0.90±0.05	41.6±0.7	1.57 ± 0.04^{c}
TG18	0.84±0.10	$31.7\pm0.2^{a,b}$	$1.85\pm0.06^{d,e,f}$
TG12:TG16 (20:80)	0.83±0.13	46.6±9.2	1.36 ± 0.20^{d}
TG12:TG16 (50:50)	0.86±0.14	52.2 ± 12.4^{a}	$1.23\pm0.20^{a,e}$
TG12:TG16 (80:20)	0.84±0.05	54.4 ± 11.3^{b}	$1.13\pm0.27^{b,c,f}$

Similar letters in each column signify significantly different values ($p<0.05$). Mean ± SD ($n=3$)

The release of desmopressin from the TG14 microparticles was faster than that of the larger protein lysozyme; 8.4±0.5% of lysozyme was released from TG14 microparticles after 120 min in the absence of lipase (9). The different rate of drug release can be attributed to the smaller size of desmopressin, which would provide a higher diffusivity through the particle matrix compared to lysozyme.

In the presence of lipase, the rate of drug release from TG microparticles decreased with an increase in the chain lengths of fatty acyl groups (Fig. 2, closed symbols). Similar results were observed for similar particles loaded with lysozyme in a recent work (9). The accelerated drug release from the TG microparticles with shorter chain length TG is due to a faster hydrolysis rate of the TG, which is supported by the data of released FA during the lipolysis. The release of FA (Fig. 2, open symbols) follows the overall trend of the desmopressin release profiles. The particles therefore exhibit a lipase-mediated release of desmopressin, similar to previous observations of lysozyme release from TG microparticles (9).

Peptide and protein drugs entering the GIT will be subject to degradation by proteases. To evaluate the protection afforded by the different SLM, digestion experiments were also conducted in the presence of both lipase and α-chymotrypsin. Limited information is available about the intestinal α-chymotrypsin activity, therefore the amount of α-chymotrypsin was chosen to degrade more than 50% of the added desmopressin during the two hours of lipolysis. The microbial lipase is also a substrate of α-chymotrypsin and some degradation is likely to occur during the experiments. However, due to the excess lipase activity, this did not affect the lipolysis process as the amount of FA hydrolyzed was similar with and without α-chymotrypsin (data not shown).

The amount of desmopressin degraded during the lipolysis of the TG microparticles in the presence of protease was compared to the data for the degradation of non-encapsulated desmopressin (Fig. 3). For the three formulations *i.e.* TG14, TG16, and TG18 microparticles, 75.4±8.7%, 54.3±0.9% and 7.7±0.9% of the desmopressin was degraded compared to non-encapsulated desmopressin (using Eq. 3), respectively. Protection against degradation is one of the

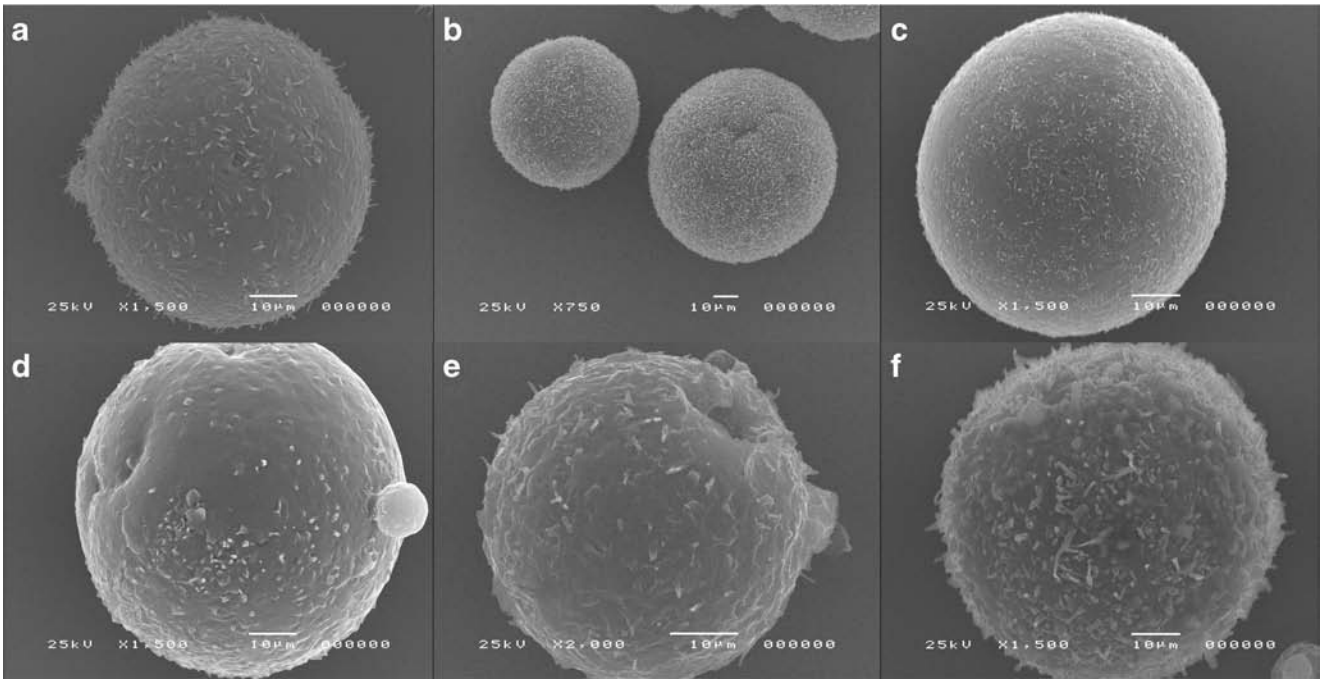

Fig. 1 SEM-micrographs of solid lipid microparticles at 1500× magnification unless otherwise specified. (**a**) TG14 microparticles, (**b**) TG16 microparticles (750× magnification), (**c**) TG18 microparticles, (**d**) TG12:TG16 (20:80) microparticles, (**e**) TG12:TG16 (50:50) microparticles (2000× magnification), (**f**) TG12:TG16 (80:20) microparticles. Size bars: 10 μm.

important factors for oral peptide formulations, but it is also important to maintain a high peptide drug concentration in the intestine to promote absorption. To evaluate the amount of desmopressin available for absorption during the entire 2 h lipolysis experiments, the AUC of the desmopressin release profile in the presence of protease and lipase was calculated and compared to the value for the incubation of desmopressin with protease (Table III). These data show that even though desmopressin is degraded to a higher extent when it is present in solution, it still provides the highest availability of desmopressin during the 2 h of the release study under the selected conditions.

The degradation constant for desmopressin in the presence of α-chymotrypsin was found to be 0.0106 min^{-1} by incubating non-encapsulated desmopressin in the lipolysis medium containing both lipase and α-chymotrypsin for 120 min. With this degradation constant the theoretical release profiles of desmopressin in the presence of protease, was calculated using the Eq. (2) based on the release profiles of desmopressin in the

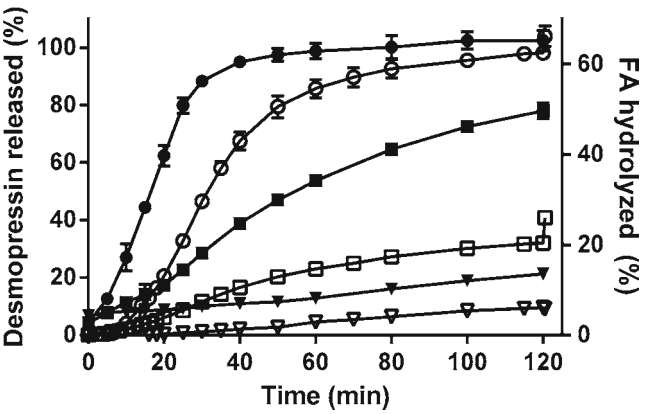

Fig. 2 The release of desmopressin during *in vitro* lipolysis (*closed symbols*) and the amount of FA hydrolyzed (*open symbols*) from TG particles. The last point in the FA hydrolysis curves is after pH adjustment to 9.0. ●, TG14 microparticles; ■, TG16 microparticles; ▼, TG18 microparticles. Mean ± SD ($n = 3$).

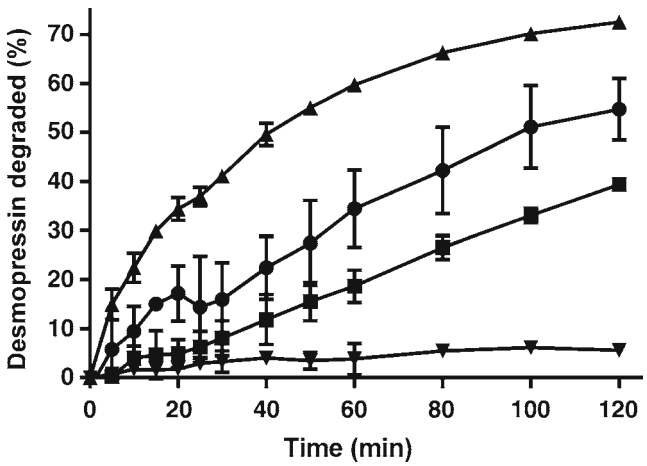

Fig. 3 Degradation of desmopressin during lipolysis studies of TG microparticles in the presence of α-chymotrypsin. ▲, non-encapsulated desmopressin; ●, TG14 microparticles; ■, TG16 microparticles; ▼, TG18 microparticles. Mean ± SD ($n = 3$). Data express how much of the total desmopressin dose is degraded in the lipolysis experiment.

Table III The AUC and the Desmopressin Amount Available for Absorption After the Release of Desmopressin from the SLM and Non-Encapsulated Desmopressin in the Presence of Protease[1]. The Availability of Non-Encapsulated Desmopressin was Set at 100%

Formulation	AUC (% · min)	Availability (%)
TG14	5844 ± 779	94.3 ± 12.6
TG16	3106 ± 395	50.1 ± 6.4***
TG18	1055 ± 92.4	17.0 ± 1.5***
Desmopressin	6197 ± 150.4	100

[1] AUC: Determined on the release profiles of desmopressin in the presence of lipase and protease. Availability: AUC values compared to the AUC for the non-encapsulated desmopressin

*** $p < 0.001$

Statistics determines significant difference to the non-encapsulated desmopressin. Mean ± SD ($n = 3$)

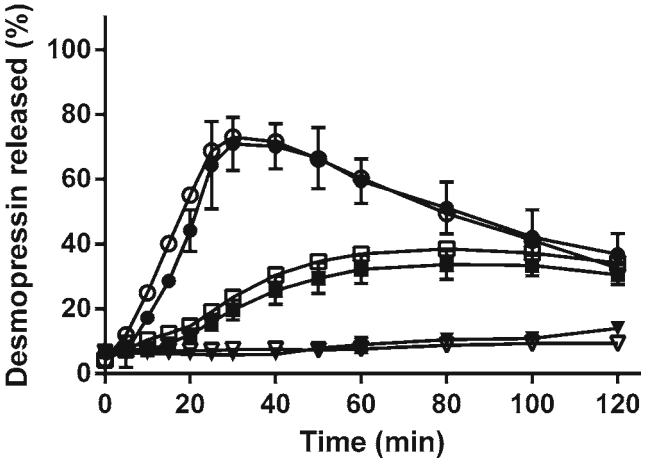

Fig. 4 Comparison of desmopressin release profiles in the lipolysis experiments in the presence of protease (*closed symbols*) and the theoretical release profiles (*open symbols*). ●, TG14 microparticles; ■, TG16 microparticles; ▼, TG18 microparticles. Mean ± SD ($n = 3$).

presence of lipase but not protease (Fig. 2). The degree of similarity of the profiles for the amount of desmopressin available in the presence of protease and the calculated profile (Fig. 4) indicates if the drug degradation is only based on lipolysis-induced release and subsequent degradation by α-chymotrypsin in the medium. The calculated theoretical release curves for the release of desmopressin from all the TG microparticles in the presence of protease followed the measured release data (Fig. 4), indicating that the presence of the TG microparticles does not affect the activity of the protease and that the protecting effect is only caused by the sustained release of desmopressin. The lipolysis model thus makes it possible to differentiate between lipolysis-mediated release and the subsequent degradation of desmopressin elucidating the role of TG in the protection of desmopressin.

Even though the TG16 and TG18 microparticles were superior in protecting desmopressin against protease degradation, the availability of desmopressin from these particles was much less than the desmopressin in solution. This is obviously caused by the incomplete release of desmopressin during the experiment, which leads to low availability for absorption in the GIT. TG14 microparticles can provide protection of desmopressin from degradation as well as a high availability for absorption. Whether a fast release and thereby high initial availability for absorption is advantageous for the absorption of desmopressin needs to be investigated *in vivo*. The information gained from this model might permit improved interpretation of *in vivo* results.

Microparticles Made from Mixtures of TG12 and TG16 Show Different Release and Protection Properties

To investigate the combined effect of the sustained release properties of TG16 microparticles and the erosion properties of TG12 observed by Schwab *et al.* (19), SLM were also prepared with mixtures of TG12 and TG16 in the ratios of 20:80, 50:50 and 80:20.

The inclusion of TG12 in the SLM significantly accelerated the release of desmopressin in the presence of lipase (Fig. 5a) compared to TG16 microparticles (Fig. 2). Desmopressin was released completely after 15 min of lipolysis of TG12:TG16 (20:80) particles. Particles with a TG12 fraction of 50% and 80% resulted in even faster release rates with all desmopressin released after 5 min of lipolysis. The accelerated release of desmopressin is primarily attributed to an increased lipolysis rate, because the FAs were hydrolyzed faster from these SLM (Fig. 5a) than from pure TG16 particles (Fig. 2). The faster lipolysis rate is driven by the fast degradation of TG12 compared to that of the TG16, therefore increased amounts of TG12 in the particles results in faster desmopressin release. Additionally, minimal lipolysis was required to afford full release of desmopressin from the particles, which could be caused by destabilization of the lipid matrix upon lipolysis. In the absence of lipase, 22.1 ± 11.3% and 29.9 ± 12.1% of desmopressin was released from the particles with 20% and 50% TG12, respectively, whereas 61.7 ± 14.7% of desmopressin was released from particles with 80% TG12. Rapid erosion of TG12 (19) could explain the instability of lipid blend particles with larger amounts of TG12, which caused a higher degree of drug diffusion from the particles.

The particles with 50% and 80% TG12 showed increased protection of desmopressin against α-chymotrypsin mediated degradation, even though desmopressin was released from these particles faster than from the particles with 20% TG12 (Fig. 5b). It has been reported previously that monohexanoin could protect desmopressin against degradation by pancreatic juice *in vitro* (22) which could be a similar effect to what is observed for TG12 in these experiments. However, the very fast release of desmopressin from the blend microparticles still

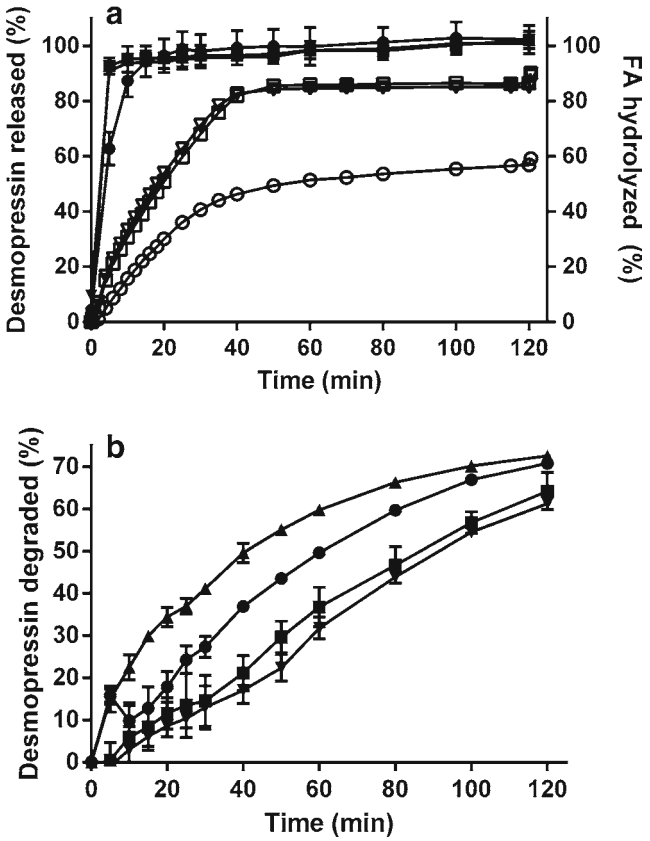

Fig. 5 (**a**) Release of desmopressin (*closed symbols*) during the *in vitro* lipolysis and the amount of FA hydrolyzed (*open symbols*) and (**b**) degradation of desmopressin in the presence of lipase and protease from microparticles made of TG blends. The last point in each of the FA hydrolysis curves is after pH adjustment to 9.0. ▲, non-encapsulated desmopressin; ●, TG12:TG16 (20:80) microparticles; ■, TG12:TG16 (50:50) microparticles; ▼, TG12:TG16 (80:20) microparticles. Mean ± SD ($n=3$).

make them inferior with regards to the protection of desmopressin from degradation as compared to the pure TG16 and TG18 microparticles ($p<0.05$). Degradation values of 97.6±0.7%, 88.5±6.1% and 84.4±1.0% were obtained from the particles composed of TG12:TG16 (20:80), (50:50), and (80:20), respectively. The degradation decreased with increasing amounts of TG12 in the particles, but no significant difference was found between the SLM with 50% TG12 and 80% TG12 ($p<0.05$). The SLM with 50 and 80% TG12 provided significantly higher desmopressin availability than the particles with 20% TG12 ($p<0.001$). For the particles with 80% TG12 a significantly higher value than non-encapsulated desmopressin was obtained ($p<0.05$, Table IV).

When comparing the similarity between the calculated and the measured release curves of desmopressin in the presence of protease, the measured release profile of desmopressin from the particles with 20% TG12 was similar to its calculated release profile, whereas the calculated release profile underestimated the measured release of desmopressin from

Table IV The Desmopressin Available for Absorption During Lipolysis of SLM and Free Desmopressin in the Presence of Protease

Formulation (w/w)	Availability (%)
TG12:TG16 (20:80)	86.0±12.8*
TG12:TG16 (50:50)	110.0±3.5
TG12:TG16 (80:20)	116.6±5.3*
TG12 + TG14	122.4±1.1**
TG12 + TG16	122.8±7.0**
TG12 + TG18	66.3±4.2***
Desmopressin	100

*$p<0.05$, ** $p<0.01$, *** $p<0.001$

Statistics determines significant difference to the non-encapsulated desmopressin

Mean ± SD ($n=3$)

the particles with 50% and 80% TG12 (data not shown). This indicates that physical protection is not the only determinant of the amount of desmopressin available. It is therefore plausible that TG12 protects desmopressin from proteolytic degradation by inhibition of the protease or by interacting with desmopressin which is also reflected in the availability of desmopressin for absorption (Table IV).

Drug-Free TG12 Microparticles Influence the Release and Degradation of Desmopressin from the SLM

When drug-free TG12 microparticles were mixed with drug-loaded SLM in the lipolysis studies, the release of desmopressin was accelerated from all drug loaded TG microparticles (Fig. 6a). The effect was more profound for TG14 and TG16 microparticles, whereas the TG18 microparticles still showed sustained release profiles with 74.1±2.0% of desmopressin released after the 120 min of lipolysis. One of the possible reasons for the accelerated release of desmopressin is the enhanced wetting of the SLM by lipolysis products of TG12, *i.e.* monolaurin and lauric acid, which have high surface activities (23,24), thereby providing increased surface area for the lipase to interact. Fatty acids, surfactants and liquid crystalline phases are also known to interact with lipids (25,26). The release of desmopressin from the TG18 particles was fast during the digestion of the drug-free TG12 microparticles, but the release of desmopressin was stopped immediately after the lipolysis reaction slowed down (Fig. 6a). This strongly indicates an immediate effect of the produced lipolysis products on the release of desmopressin from the TG18 microparticles. It was therefore hypothesized that it might be possible to control the release of desmopressin from TG18 microparticles by varying the amount of TG12. Studies on the release of desmopressin in the presence of 10 times less TG12 showed a slower release profile (Fig. 6a) but still faster than that without TG12 (Fig. 2) which exemplifies the

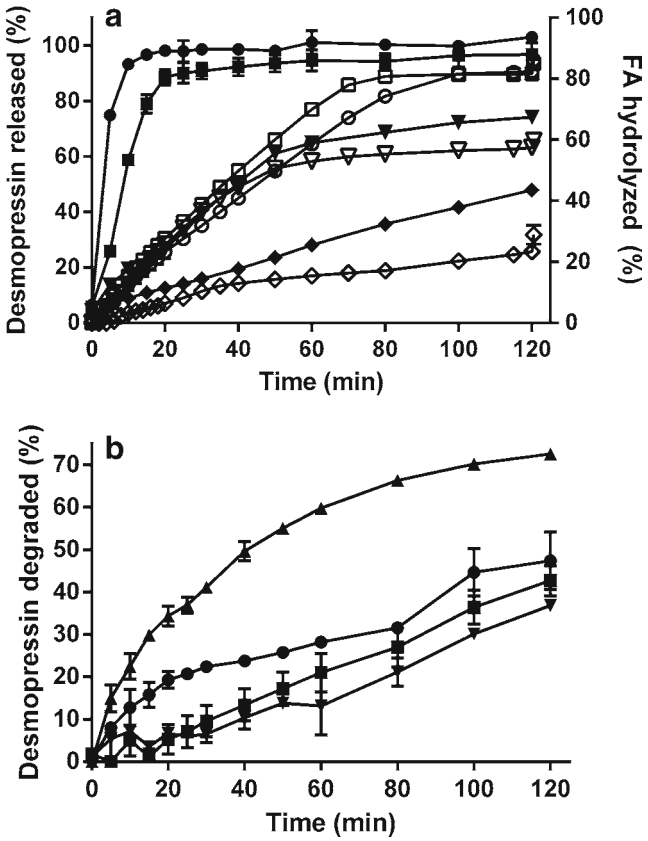

Fig. 6 (**a**) Release of desmopressin (*closed symbols*) during *in vitro* lipolysis in the presence of lipase and the amount of FA hydrolyzed (*open symbols*) and (**b**) degradation of desmopressin in the presence of lipase and protease from 100 mg drug-free TG12 microparticles mixed with 100 mg drug-loaded SLM unless otherwise specified. The last point in the FA hydrolysis curves is obtained after pH adjustment to 9.0. ▲, non-encapsulated desmopressin; ●, drug-free TG12 microparticles + TG14 microparticles; ■, drug-free TG12 microparticles + TG16 microparticles; ▼, drug-free TG12 microparticles + TG18 microparticles; ♦ drug-free TG12 microparticles (10 mg) + TG18 microparticles (100 mg). Mean ± SD ($n=3$).

possibility of altering the release of desmopressin from TG18 particles by varying the amount of TG12.

The combination of drug-free TG12 microparticles with SLM increased the protection of desmopressin (Fig. 6b) which is probably due to the increased amount of TG12 in the lipolysis study. Desmopressin degradation values of 65.3± 9.4%, 58.9±4.9%, and 51.7±0.7% were obtained for TG14, TG16, and TG18 microparticles, respectively. More desmopressin was available for absorption ($p<0.01$) from the TG14 and TG16 microparticles in the presence of the TG12 microparticles compared to the non-encapsulated desmopressin (Table IV).

A combination of drug-free TG12 microparticles and TG18 microparticles could be relevant for obtaining simultaneous sustained release and decreased degradation of desmopressin. Because the release of desmopressin from TG18 microparticles seems to follow the degradation of drug-free TG12 microparticles, the control of desmopressin release by altering the amount of drug-free TG12 microparticles could be an interesting strategy. If degradation of TG12 is synchronized with desmopressin release from TG18 microparticles it would also ensure that degradation inhibiting compounds are generated from TG12 degradation during the entire release period. Furthermore, lauric acid is a known permeability enhancer in the intestines which could be another advantage for the peptide formulation (27,28).

To summarize, the lipolysis products of TG12 affect the desmopressin release from other TG microparticles and have a protecting effect on the enzymatic degradation of desmopressin. This makes TG12 a very interesting lipid to use for oral lipid formulations containing peptides and proteins.

CONCLUSIONS

Encapsulation of desmopressin in SLM made of saturated TGs provides sustained release of desmopressin during *in vitro* digestion in biorelevant medium containing bile salt and phospholipid. The medium-chain triglyceride, TG12, accelerates the release of desmopressin from SLM both as separate particles and as a part of a lipid melt with TG16. Furthermore, the α-chymotrypsin-promoted degradation of desmopressin is reduced in the presence of TG12. The extended digestion model established in this study provides the opportunity to simultaneously simulate the effect of proteolytic degradation of peptides and lipolysis of lipid excipients by lipase. By developing biorelevant *in vitro* models capable of distinguishing the relevant processes in the GIT, it is possible to gain a better mechanistic understanding of critical factors for oral delivery of peptide or protein drugs.

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