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Oral peptide delivery by tetraether lipid liposomes

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ABSTRACT

The aim of this study is to improve of oral peptide delivery by a novel type of liposomes containing tetraether lipids (TELs) derived from archaea bacteria. Liposomes were used for the oral delivery of the somatostatin analogue octreotide. TELs were extracted from *Sulfolobus acidocaldarius* and subsequently purified to single compounds. Liposomes were prepared by the film method followed by extrusion. Vesicles in size between 130 and 207 nm were obtained as confirmed by photon correlation spectroscopy. The pharmacokinetics of radiolabeled TELs in liposomes was investigated after oral administration to rats. 1.6% of the applied radioactivity in fed and 1.5% in fasted rats was recovered in the blood and inner organs after 2 h, while most of the radioactivity remained in the gastro-intestinal tract. After 24 h the percentage of radioactivity in inner organs was reduced to 0.6% in fed rats, respectively 1.0% in fasted animals. Several liposomal formulations containing dipalmitoyl phosphatidylcholine (DPPC) and TELs in different ratios were loaded with octreotide and orally administered. Liposomes with 25% TEL could improve the oral bioavailability of octreotide 4.1-fold and one formulation with a cationic TEL derivative 4.6-fold. TEL-liposomes probably act by protecting the peptide in the gastro–intestinal tract.

1. Introduction

Recent advances in biotechnology provide tools to manufacture peptides on a large scale and the number of therapeutically useful peptide-based drugs is continuously increasing (Kumar et al., 2006). Peptides and peptidomimetics have a broad range of potential clinical benefits, with application in some of the most prevalent diseases, such as antibiotic/antifungal and antiviral indications, immune system disorders, cancer, cardiovascular indications and neurological disorders (for review see Groner, 2009). In addition, some are of interest in diagnostic applications, e.g. radionuclide labeled somatostatins (Bal et al., 2010).

Especially for long and repeated therapeutic use, oral delivery is by far the most convenient route for drug delivery. But the development of formulations for oral administration of peptide drugs is rendered more difficult for several reasons. Peptides may be degraded pre-systemically in the gastro–intestinal tract (GIT) and the low fraction reaching the intestinal wall is mostly poorly absorbed due to the high molecular weight and hydrophilicity of peptides (Fricker and Drewe, 1996; Hamman et al., 2006). Only a few therapeutically relevant peptide drugs are available as oral dosage forms, e.g. cyclosporine A (microemulsion) or desmopressin (Table 1). Due to a bioavailability below 1% for most proteins, a direct systemic delivery by intravenous or subcutaneous injection remains to be the first choice of administration despite obvious disadvantages (Ziv and Bendayan, 2000). Patient compliance is rather low, especially in case of chronic diseases, self-administration remains difficult and there is a certain risk of infections by inappropriate handling of the injection device. On the other hand oral drug delivery is normally associated with a high compliance and effective drug delivery systems would enable peptides to be delivered via the intestinal route.

Several strategies towards an oral peptide delivery have been suggested, for instance use of absorption enhancers like surfactants and small molecule carriers, enzyme inhibitors, chemical modifications and use of particular systems, e.g. liposomes, solid lipid nanoparticles, polymeric nanoparticles or PEG-chitosan nanocapsules (des Rieux et al., 2006; Fricker and Drewe, 1996; Liang and Yang, 2005; Malkov et al., 2005; Patel et al., 1982; Stoll et al., 2000).

Initial approaches to the use of liposomes for oral peptide delivery were not very encouraging mostly due to a poor reproducibility of results (Chiang and Weiner, 1987b; Patel et al., 1982). Nevertheless, liposomes have some important advantages over other delivery systems as they are well characterised, have a good biocompatibility and high versatility (Jesorka and Orwar, 2008).

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Table 1
Comparison of the theoretical proton signal intensities in the ^{1}H NMR of the different carbon groups for GCTE with four, five or six cyclopentyl moieties to the measured signal intensities of the purified GCTE.

Group	Relative proton signal intensity			
	4 Rings	5 Rings	6 Rings	Measured
$-OCH_2$	24	24	24	26.36
$-CH_3$	36	33	30	36
$-CH/-CH_2$	108	109	110	120.18
Signal ratio				
$(-CH/-CH_2)/-OCH_2$	3.00	3.30	3.67	3.34
$(-CH/-CH_2/-CH_3)/-OCH_2$	6.00	5.92	5.83	5.92

Moreover, mucoadhesive liposomal systems prepared by coating lipid suspensions with polymers, such as chitosan or carbopol, have shown some success in intestinal absorption of peptide drugs (Takeuchi et al., 2005; Thongborisute et al., 2006). A novel approach is presented here with the use of tetraether lipid-containing liposomes. Such liposomes may offer new options for an oral delivery of peptides and protein drugs. Li et al. (2010) recently showed that liposomes made of the polar lipid fraction of *Sulfolobus acidocaldarius* can deliver insulin orally. TELs are abundantly present at high diversity from both bacterial and archaeal sources (Lo and Chang, 1990; Schouten et al., 2007). The membrane of hyperthermophilic archaeobacteria is predominantly composed of isoprenoid glycerol dibisphytanyl glycerol tetraethers with additional cyclopentyl moieties. Quantities of these moieties in the archaeal cell membrane differ with growth conditions and increase with the environmental temperature. The lipids glycerylcaldityl-tetraether (GCTE) and diglyceryl-tetraether (DGTE) with an average number of four to six cyclopentyl rings have been isolated from *S. acidocaldarius* and the cationic lipid AF-1 has been semi-synthetically obtained from DGTE (Fig. 1). *S. acidocaldarius* grows at temperatures between 50 °C and 100 °C mostly under acid conditions, demanding a cell membrane, which is stable under these harsh conditions (Lo and Chang, 1990). Due to the ether bonds and the unsaturated alkyl chain, TELs are less susceptible to hydrolysis and oxidation than normal ester phospholipids. A mixture of TELs with conventional phospholipids results in a combination of mono- and bilayers, whereby the membrane-spanning character and the rigidity of TELs lead to a membrane stabilisation. However, their glass transition temperature below 0 °C allows an easy production and handling at room temperature of liposomes containing these lipids (Gambacorta et al., 1995). Altogether, the properties of TELs make them interesting for use in liposomes, which have to face aggressive conditions like in the gastro–intestinal tract.

In this study, we evaluated the pharmacokinetics of radiolabeled DGTE in liposomes after oral application and tested the performance of several liposomal formulations as oral delivery systems for the somatostatin analogue octreotide in rats. Somatostatin is a 14 amino acid peptide hormone inhibiting pancreatic exocrine and endocrine secretion. Its clinical application is limited by its very short half life, making continuous intravenous infusion necessary. Octreotide is an 8 amino acid synthetic analogue of somatostatin possessing similar pharmacological effects but a significantly prolonged plasma half life. It is used for a variety of indications such as acromegaly, gastro–intestinal disorders and psoriasis (Batershill and Clissold, 1989; Prommer, 2008). It has been stabilised against proteolytic degradation in gastrointestinal fluids (Pless et al., 1986) making oral administration feasible (Köhler et al., 1987; Fuessl et al., 1987; Drewe et al., 1993; Fricker et al., 1991; Haeberlin et al., 1996). However, due to low bioavailability (<0.3%) it is only given by intravenous or subcutaneous administration (Arts et al., 2009; Drewe et al., 1993).

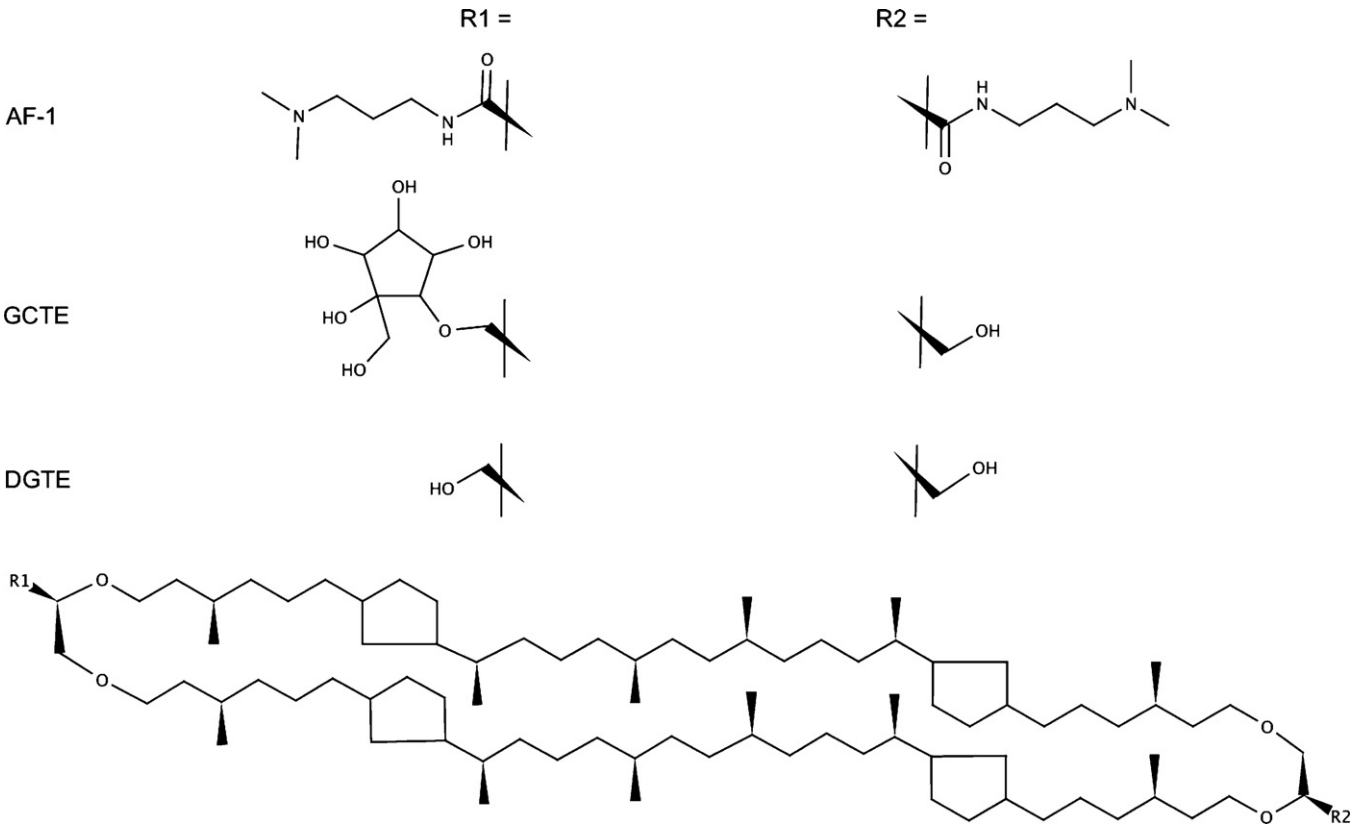

Fig. 1. Structure of the neutral TELs GCTE and DGTE and of the cationic derivative AF-1.

2. Materials and methods

2.1. Materials

Egg phosphatidylcholine (EPC) and dipalmitoyl phosphatidylcholine (DPPC) were gifted from Lipoid GmbH (Ludwigshafen, Germany). Octreotide was provided by Novartis AG (Basel, Switzerland). Cholesterol (Chol) was purchased from Sigma–Aldrich (Taufkirchen, Germany). ^{3}H-labeled DGTE was prepared by Amersham (Buckinghamshire, UK). All other chemicals were obtained in the highest purity from the usual commercial sources.

2.2. Isolation and purification of GCTE and DGTE

Growth of cells and extraction of lipids was performed similar to the methods described previously by Lo et al. with some small changes (Lo et al., 1989). Briefly, *S. acidocaldarius* was grown heterotrophically in a 78 °C fermenter at pH 3.3. Subsequently, cells were separated from growth medium and lyophilised. The lipids were isolated from the lyophilised biomass by Soxhlet extraction with a mixture of chloroform/methanol (2:1) for 6 days. Solvent from the extract was removed and the residue was dissolved in a mixture of chloroform, methanol and hydrochloric acid. The mixture was heated to reflux for 3 days to cleave the natural lipid headgroups. Finally, lipids were extracted at room temperature with chloroform/methanol (2:1) from the water phase.

GCTE was separated from other lipids by a two step silica gel chromatography with chloroform/methanol (9:1) as mobile phase, followed by a Sephadex® LH20 chromatography with chloroform/methanol (1:1). GCTE was separated from residual impurities by recrystallisation in acetone. Solvent from the GCTE fraction was removed and the lipid was dissolved in a sufficient amount of dichloromethane. Subsequently, six times the volume of acetone at 40 °C was added and GCTE was crystallised at 7 °C. This step was performed twice.

2.3. Lipid analyses

^{1}H NMR spectrum of the TEL was acquired using a Bruker Avance II 400 system (Bruker BioSpin GmbH, Rheinstetten, Germany) with a 9.4 Tesla magnet. Samples were dispersed in D_2O with tetramethylsilane as an internal reference and measurements were carried out at 25 °C. The spectrum was received at 399.89 MHz with 6 μs ^{1}H pulses for free-induction decays and 4 s pulse delay.

For the IR spectrum a GCTE film was sandwiched between to NaCl plates and spectrum was acquired using a Nicolet Avatar 320 FT-IR spectrometer (Thermo Fisher Scientific GmbH, Dreieich, Germany).

Desorption chemical ionisation mass spectrometry was carried out by the use of a Finnigan TSQ 700 (Thermo Finnigan MAT, Bremen, Germany) system with a quadrupole mass filter and isobutane as reagent gas.

2.4. Liposome preparation

All liposomal formulations were prepared by the film method according to Bangham et al. (Bangham et al., 1965). Briefly, a mixture of host-lipids, e.g. EPC or DPPC, TEL and other lipids, e.g. cholesterol, were dissolved in chloroform/methanol (8:1) and subsequently the solvent was removed in a rotary evaporator. The resulting lipid film was dried for additional 20 min at 20 mbar. Finally, H_2O and Teflon-beads were added to the film and the flask was rotated for 12 h at 45 °C to give a white homogenous suspension of multilamellar vesicles (MLVs).

With respect to the TEL pharmacokinetic study, 0.75% of ^{3}H-DGTE was added to an EPC:GCTE 4:1 lipid mixture and a lipid film was prepared as described above. The MLVs were extruded 19 times through a 100 nm membrane using a LiposoFast extruder (Avestin, Ludwigshafen, Germany).

For the bioavailability study, the MLV suspension was frozen in liquid nitrogen and thawed in a water bath at 40 °C. This procedure was repeated twice and resulted in the formation of large lipid aggregates. After centrifugation at 25,000 × *g* for 60 min (Sigma 4K15, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) the lipid aggregates were collected in the pellet. The supernatant was discarded and the pellet was resuspended in water. Extrusion of liposomes was performed using a 10 ml Lipex™ (Northern Lipids, Burnaby, BC, Canada) extruder equipped with nucleopore filter membranes (Whatman, Maidstone, UK) and heated to a temperature of 50 °C. Driven by a pressure of max. 40 bars the liposomes were extruded three times through a 400 nm pore size filter and ten times through a 200 nm pore size filter. A peptide stock solution of octreotide acetate in water was prepared and a required amount of the solution was gently mixed with the liposome suspension. Teflon beads were added and the mixture was frozen in liquid nitrogen. The frozen formulation was lyophilised for 12 h at −25 °C and 1 mbar (main drying) and for additional 2 h at 25 °C and 0.5 mbar (secondary drying). After lyophilisation the vials were sealed and stored at 4 °C. In order to rehydrate the liposomes, a volume of 100 μl 0.1% acetic acid was added to a formulation containing 30 μmol total lipid to give an initial lipid concentration of 300 mM. Complete rehydration was achieved by vortexing. The liposomal dispersion was then diluted by a factor of ten with phosphate buffered saline pH 7.4 (PBS) (NaCl 137 mM, KCl 2.7 mM, KH_2PO_4 1.5 mM, $Na_2HPO_4{\cdot}2H_2O$ 8.1 mM). The suspensions were centrifuged at 25,000 × *g* for 20 min (Sigma 4K15, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) to separate the free peptide in the non-liposomal excess volume from the encapsulated peptide associated with the liposomes, The liposomes were collected in the pellet, while the free peptide remained in the supernatant. The pellets were diluted to the initial volume with buffer and the washing procedure was repeated twice.

2.5. Liposome analyses

The particle size was checked using a Nicomp particle sizing system (380 ZLS, Nicomp Inc., Santa Barbara, CA, USA) in the intensity mode. Phospholipid content was determined using an enzymatic phosphorous assay described by Rouser et al. (Rouser et al., 1970). The content of DPPC was measured by quantification of orthophosphate that was liberated from the lipid during oxidation. The content of GCTE was measured by HPTLC after extraction of formulation samples with $CHCl_3$/MeOH (8:1).

Liposomal (encapsulated) and the free (non-encapsulated) octreotide in the samples was determined by HPLC. A sample of 20 μl formulation was diluted with 180 μl PBS and centrifuged at 25,000 × *g* for 20 min (Sigma 4K15, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). The quantity of free octreotide was determined in the supernatant and the amount of encapsulated octreotide in the pellet after dissolving with 180 μl Zwittergent solution (20% w/v in H_2O).

Octreotide was analysed using a HP 1090 Liquid Chromatograph system (Hewlett Packard GmbH, Waldbronn, Germany) with a GROM SiL 120 Butyl-1 ST, 5 μm, 20 × 4.6 mm precolumn and a GROM SiL 100 ODS-0 AB, 5 μm, 250 × 4.6 mm column at 25 °C. Flow of the solvent acetonitrile/phosphate buffer (Na_2HPO_4 16.00 mM, NaH_2PO_4 1.75 mM, pH 7.4) (1:1) was kept constant at 1 ml/min throughout the run and the concentration of octreotide was deter-

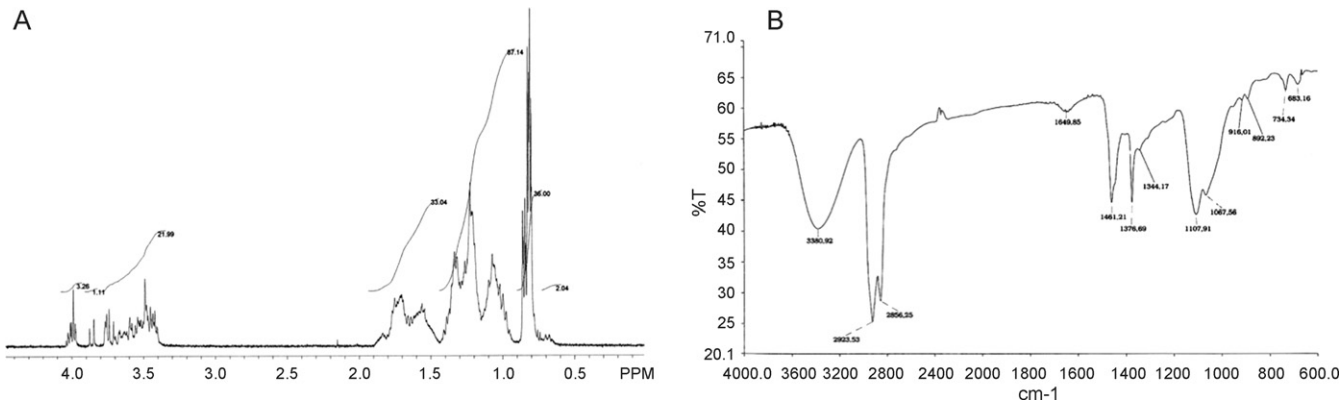

Fig. 2. ^{1}H NMR spectrum (A) and IR spectrum (B) of purified GCTE.

mined by comparing the UV absorption at 210 nm to a calibration curve.

2.6. Animal studies

All animal studies were performed according to the guidelines of the local authorities using male Wistar rats (body weight 250–280 g).

For the TEL pharmacokinetic study, the liposomes were applied orally by gavage to male rats (fed state). The dose was 7.5 mg lipid/kg rat, corresponding to a radioactivity of 188 μCi/kg rat. A second group of rats was kept without food, but with free access to water for 12 h before the experiment. To this group a dose of 2.5 mg lipid/kg rat, corresponding to a radioactivity of 62 μCi/kg rat was applied.

After administration, blood samples were taken after 0.5, 1, 2, 4, 8 and 24 h. Animals of each group were sacrificed after 2 h and the other two animals after 24 h. The animals were sectioned, organs were homogenised in saline and the specific radioactivity of all relevant organs was assayed by scintigraphy (2000 CA Tri-Carb, Packard, Zürich, Switzerland). Additionally, the stomach content, the intestinal content and the faeces were analysed.

To determine the bioavailability of octreotide, rats were kept without food, but with free access to water for 12 h before the experiment. 500 μl of each formulation corresponding to approximately 100 μg octreotide were administered orally by gavage to six rats. In a control experiment octreotide was administered in PBS. Blood samples were taken from the retro-orbital venous plexus with final sacrification, in an overlapping manner to have time points at 0.5, 1, 2 and 4 h with five rats each. The blood samples were collected in heparinised vials and centrifuged at 1000 × *g* for 10 min in an Eppendorf centrifuge 5415 C (Eppendorf, Hamburg, Germany). Plasma was kept frozen at −80 °C until the concentration of octreotide was determined by use of a specific radioimmunoassay (ANAWA, Basel, Switzerland) as described before (Michael et al., 2000). The rabbit antiserum recognised only the intact peptide with a very low cross-reactivity to peptide fragments, somatostatin-14 or somatostatin-28. The area under the plasma curve (AUC) was estimated using the trapezoidal rule.

2.7. Statistical analysis

All values are presented as means ± SEM. Control and treatment groups were compared by one-way Student's *t*-test. Differences were considered significant at $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Plots and statistical analysis were made using the software Prism® (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Lipid analyses

We first characterized the TEL, which was incorporated into the liposomes. The ^{1}H NMR spectrometric data of GCTE corresponds to that reported by Lo et al.: δ 0.7–0.9 (–CH_3), 0.95–1.4 (–CH, –CH_2), 1.45–1.9 (cyclopentyl –CH), 3.4–4.05 (–O–CH, –O–CH_2) (Fig. 2A) (Lo et al., 1989). To allow an estimation of the average quantity of cyclopentyl moieties in the GCTE mixtures the relation of the proton signal intensities of the different groups was compared to the theoretical ratio (Table 2). Results indicate that GCTE with five pentyl rings is the predominant derivative.

IR-data are also in good agreement to the spectral data reported (Lo et al., 1989): one broad band at 3380 cm^{-1} (–OH), two strong peaks at 2924 and 2856 cm^{-1} (–CH, –CH_2 and CH_3), peaks at 1461 and 1377 cm^{-1} (–CH_2, respectively –CH_3), a broad peak at 1108 cm^{-1} and a smaller at 1068 cm^{-1} (–C–O (ether) and –C–O (hydroxyl)) (Fig. 2B).

The mass spectrum shows a signal group around the base peak at 1454 amu. It corresponds to the M^+ peak of GCTE with five cyclopentyl moieties and GCTE with other quantities of pentyl rings (mainly from four to six). Also the $[M+1]^+$ and $[M+2]^+$ peaks of the different GCTE molecules contribute to this signal group. Furthermore, fragments, missing the polyhydroxyl headgroup, were visible at 1292 amu.

Table 2
Recovery of the administered radioactivity in different compartments 2 h and 4 h after administration to fasted or fed rats. Each value represents the mean recovery in % (means ± SEM, $n = 2$).

	Time point (h)	Recovery of administered radioactivity (%)			
		Organs/blood	GIT	Faeces	Urine
Fed	2	1.58 ± 0.64	90.12 ± 3.84	0.01 ± 0.00	0.04 ± 0.06
	24	0.62 ± 0.52	72.19 ± 1.58	0.52 ± 0.18	0.13 ± 0.09
Fasted	2	1.46 ± 0.44	91.47 ± 1.19	0.01 ± 0.01	0.03 ± 0.01
	24	0.97 ± 0.01	51.00 ± 0.09	32.20 ± 0.57	0.17 ± 0.10

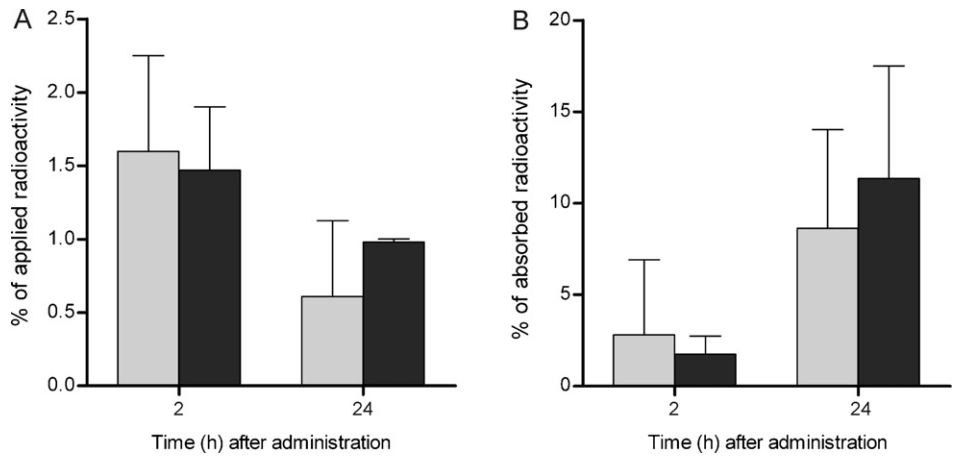

Fig. 3. Recovery of applied radioactivity from inner organs and blood stream in fed rats (light grey) and fasted rats (dark grey) after 2 h and 24 h (A) and amount of radioactivity excreted via the urine in fed rats (light grey) and fasted rats (dark grey) after 2 h and 24 h (B). Each value represents the mean of two samples in % ± SEM.

3.2. Disposition of TELs after oral administration

We tried to follow the fate of orally administered TELs by determining the disposition of radioactivity after administration of ^{3}H-labeled DGTE to fed and fasted rats by gavage. Distribution of DGTE-associated radioactivity in different body compartments after 2 and 24 h is shown in Table 2. The portion of radioactivity found in the plasma was negligible for all time points. For both, the fed and the fasted state, most of the radioactivity was recovered from the gastro–intestinal tract (GIT) 2 h after administration. It was nearly equally distributed between stomach and intestine. After one day, radioactivity could only be detected in the intestine (fed state) or in the intestine and the faeces (fasted state) (data not shown). The fraction of radioactivity in blood and organs after 2 h was around 1.5% and decreased further with time, whereas the quantity of radioactivity in the urine increased (Fig. 3).

3.3. Liposome characterisation

The different formulations loaded with octreotide were between 130 and 207 nm in size indicating the successful formation of liposomes (Table 3). Mass ratio of octreotide to lipids varied with the formulations, where liposomes with the cationic lipid AF-1 showed the lowest encapsulation efficiency (1.2% (m/m)) and the formulation with DPPC:GCTE 2:1 the highest (13.0% (m/m)).

3.4. Pharmacokinetics of octreotide after oral administration

Peroral administration of octreotide to rats resulted in significantly increased plasma concentrations as compared to administration of the peptide in aqueous solution. The plasma concentration of octreotide reached its maximum after 1 h for most of the formulations, as can be seen in Fig. 4 for representative formulations. Free octreotide and liposomes containing the cationic TEL derivative AF-1 led to a maximum plasma concentration already after 0.5 h.

Not all formulations enhanced the bioavailability of octreotide. However, EPC:Chol 2:1 and DPPC:GCTE 2:1 liposomes increased the AUC of octreotide about 2.5-fold and DPPC:GCTE 3:1 and DPPC:GCTE:AF-1 12:3:1 liposomes increased the bioavailability of octreotide more than 4.0-fold (Fig. 5). A ratio of DPPC to GCTE of 3:1 showed the best performance compared to formulations with a higher or lower GCTE quantity. It appeared, that the addition of cholesterol to the TEL liposomes had no significant effect on the bioavailability enhancement.

Table 3
Mean diameters of different liposomal formulations used for the bioavailability study and mass ratio of octreotide to total lipid (means ± SEM, $n = 3$).

Liposomal formulation	Size (nm)	Octreotide/lipid ratio (m/m %)
EPC:Chol 2:1	130 ± 17	8.7
DPPC:GCTE:Chol 12:1:6	148 ± 14	4.0
DPPC:GCTE 4:1	156 ± 25	8.1
DPPC:GCTE:Chol 12:3:2	164 ± 55	2.6
DPPC:GCTE 3:1	207 ± 62	7.1
DPPC:GCTE 2:1	162 ± 18	13.0
DPPC:GCTE:AF-1 12:3:1	133 ± 23	1.2

4. Discussion

The formation of liposomes containing mixtures of different archaeobacterial lipids has been described in the literature (Chang, 1994; Lelkes et al., 1983; Patel et al., 2000; Sprott et al., 1996). However, to our knowledge this is the first study using highly purified TELs with a defined chemical structure together with phospholipids for oral application. This is not only advantageous concerning purity and toxicity profile of the lipids but also allows a more controlled change of liposome properties. Liposomes were prepared with variable lipid mixtures up to a ratio of 1:2 GCTE:DPPC. Due to the higher rigidity of TELs compared to phospholipids a certain quantity of phospholipids is required to form stable small unilamellar vesicles. The formation of small liposomes should become more difficult with increasing quantity of TELs due to the higher membrane curvature in small vesicles (Cavagnetto et al., 1992; Lelkes et al., 1983). In this study however, GCTE showed no concentration dependent influence on size of the formed vesicles and no relation between GCTE amount and encapsulation efficiency of octreotide could be observed. The remarkably low encapsulation of the peptide in liposomes containing AF-1 could be related to electrostatic interactions of the cationic lipid and the positively charged octreotide (Beschiaschvili and Seelig, 1990).

Octreotide is preferentially absorbed in the upper jejunal tract (Fricker et al., 1991, 1992; Drewe et al., 1993) and is pharmacologically active after oral administration. However its bioavailability in rat and man is below 0.3% due to poor absorption (Drewe et al., 1993). Therefore, absorption enhancing delivery systems are highly desirable. Several approaches could be found in the literature to improve the oral bioavailability of octreotide including the use of carbohydrates, bile salts and chitosan derivatives (Drewe et al., 1993; Fricker et al., 1991; Fricker and Drewe, 1995; Michael et al., 2000; van der Merwe et al., 2004). They have in common

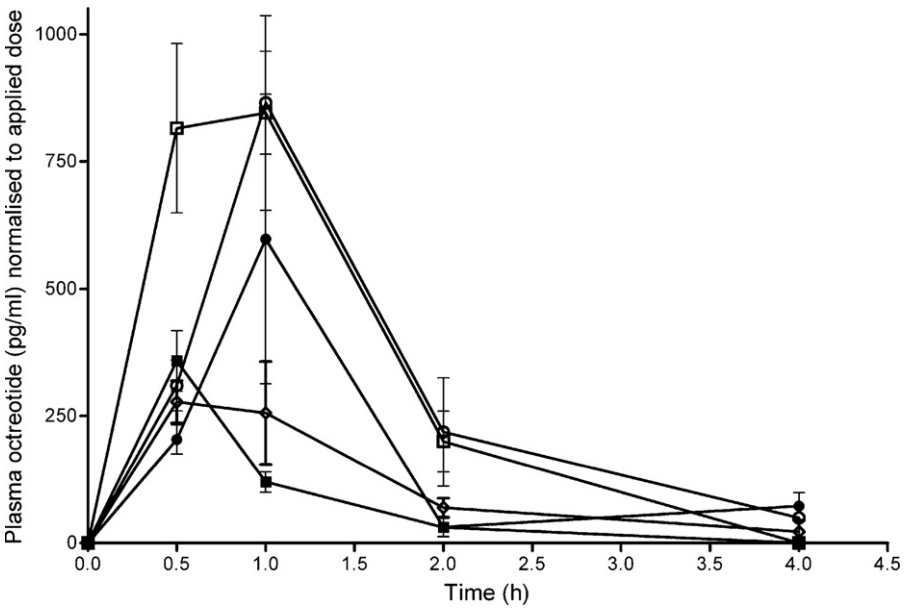

Fig. 4. Octreotide plasma concentration in pg/ml normalised to 100 μg of applied dose of free octreotide (■), EPC:Chol 2:1 liposomes (●), DPPC:GCTE:AF-1 12:3:1 liposomes (□), DPPC:GCTE 4:1 liposomes (◊) and DPPC:GCTE 3:1 liposomes (○) ($n = 6$, mean ± SEM).

to enhance permeation of the peptide through the intestinal wall, while it is still exposed to the different gastro–intestinal fluids. In the present work, we focused on the protection of octreotide during the gastro–intestinal passage and a possible permeation enhancement by uptake of liposomes into cells of the intestinal mucosa.

Dapergolas and Gregoriadis (1976) first reported the uptake of intact liposomes with an encapsulated peptide, namely insulin, into the blood system resulting in a decrease of blood glucose in diabetic rats. In the literature, uptake of intact liposomes from the intestine was controversially discussed, but Rowland and Woodley suggested that intact liposomes could pass the intestinal barrier if they are not destroyed in the stomach or intestine before (Chiang and Weiner, 1987a,b; Rowland and Woodley, 1981a,b,c). Later, the uptake of stable liposomes and other nanoparticles by Peyer's Patches could be shown (Aramaki et al., 1993; Jani et al., 1990).

In this study, the low radioactivity found in blood and organs after the oral administration of liposomes containing radio labeled DGTE suggest a low uptake of the vesicles. However, the appearance of radioactivity in organs during this study does not necessarily allow the conclusion that an uptake of intact liposomes occurs, because one cannot distinguish between uptake of intact vesicles and single lipids. The reduction of radioactivity in the inner organs after 24 h and the increase of radioactivity found in the urine indicates metabolism of the absorbed TEL followed by renal

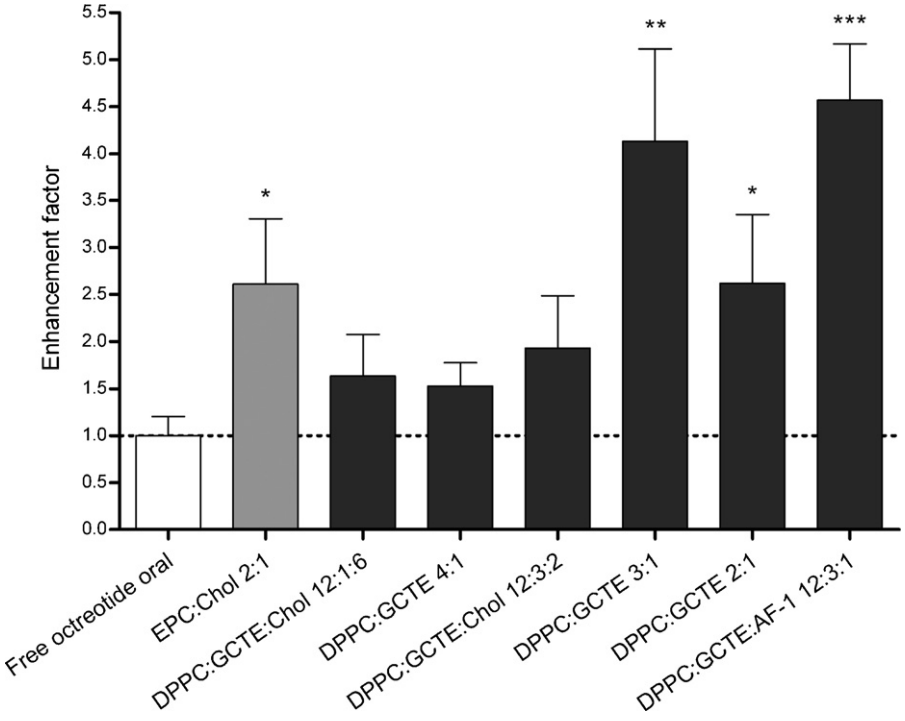

Fig. 5. Enhancement of AUC of orally administered liposomal octreotide compared to free octreotide. (Mean ± SEM, $n = 6$.)

excretion as one potential way of elimination. A renal excretion of unmetabolised compound appears unlikely due to the poor water solubility of DGTE. Biliary excretion may contribute to the elimination of DGTE, but total radioactivity in the intestine and faeces could not distinguish between radioactivity of non-absorbed and that of the excreted compound.

The different ability of the tested formulations to improve octreotide bioavailability could partly be explained by the varying stability of the tested liposomes. In addition, the cell/liposome interaction could depend on the lipid composition leading to a higher uptake or fusion rate of distinct formulations. DPPC:GCTE 2:1 liposomes should be more stable in the GIT compared to EPC:Chol 2:1 liposomes due to the higher phase transition of DPPC compared to EPC. However, the latter are more flexible and fusion with cells and permeation of the peptide might be facilitated (Elferink et al., 1997). Interestingly, the cationic formulation with AF-1 shows only a slightly better performance compared to the DPPC:GCTE 3:1 formulation with the same amount of TELs, indicating that vesicle charge has a minor influence on the absorption enhancement. Considering the low uptake of radio labeled TEL liposomes, a mechanism of absorption could be hypothesised, where octreotide can permeate into the blood system after it leaked out of liposomes adsorbed to enterocytes, whereas none or at least very few intact vesicles are taken up. In this case, the major way of absorption enhancement would rather be the protection of octreotide against the acidic conditions in the stomach and digestive enzymes in the intestine than a direct enhancement of peptide permeation by uptake of octreotide in intact vesicles.

5. Conclusion

It is possible to improve the oral bioavailability of the octapeptide octreotide by use of GCTE containing liposomes. The mode of action appears primarily to be a protection of the peptide in the intestine. The low uptake of the TELs and the fact, that in approximately one third of the population TEL producing archae can be found in the gastro-intestinal tract, suggest a good safety profile of the tested liposomes (Miller and Wolin, 1982; Sprott et al., 1999). The use of one single type of TEL lipid with defined chemical structure and not a mixture of different lipids allows the production of liposome batches in the industrial scale with steady properties and less demands on analytical methods. To further improve the bioavailability of octreotide a combination of the protective effect by TEL liposomes with methods based on the co-administration of permeation enhancers may be useful.

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