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Enhancement of nasal and intestinal calcitonin delivery by the novel Pheroid™ fatty acid based delivery system, and by *N*-trimethyl chitosan chloride

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ABSTRACT

Therapeutic peptides are highly potent and specific in their functions, but difficulties in their administration require parallel development of viable delivery systems to improve their bioavailability. In this study the potential of a novel lipid-based colloidal delivery system for improving the absorption of nasally and intestinally administered salmon calcitonin (sCT) was investigated. Two types of delivery vehicles based on Pheroid™ technology was prepared and characterized. Liposome-like bilayer vesicles had a mean diameter of 1.0 μm and microsponges were 1.6 μm. Doses of 10 IU/kg and 500 IU/kg bodyweight sCT were administered intranasally and intestinally to rats, respectively. The obtained absorption enhancement with Pheroid™ vesicles and Pheroid™ microsponges were also compared with the absorption enhancement obtained with *N*-trimethyl chitosan chloride (TMC). With the inclusion of 0.5% (w/v) TMC the maximum plasma concentration (C_{max}) of sCT increased from 72.6 ± 6.1 pg/ml to 478.5 ± 6.1 pg/ml after nasal administration. Pheroid™ vesicles and Pheroid™ microsponges increased the C_{max} values of sCT to 262.64 ± 17.1 pg/ml and 202.66 ± 28.6 pg/ml, respectively. The time to reach the maximum concentration (T_{max}) was also significantly decreased from 35 min to approximately 14 min. Intestinal administration of Pheroid™ formulations increased the C_{max} of sCT from 249.1 ± 21.5 pg/ml to 386.2 ± 45.5 and 432.1 ± 18.9 pg/ml, respectively for Pheroid™ vesicles and Pheroid™ microsponges. TMC increased the C_{max} of sCT to 738.9 ± 277.1 pg/ml. TMC and Pheroid™ technology could offer the potential to significantly improve intranasal and intestinal absorption of sCT and reduce the variability in absorption.

1. Introduction

Peptide drug molecules are highly potent and selective within a broad range of therapeutic applications (Frokjaer and Otzen, 2005). Calcitonin is a cyclic polypeptide of 32 amino acids which plays an important role in the regulation of calcium homeostasis. It is a potent inhibitor of osteoblastic bone resorption and is widely used in the treatment of osteoporosis and Paget's disease (Sexton et al., 1999). Salmon calcitonin (sCT) is the most potent of the calcitonins available and is well tolerated and clinically effective. The currently available treatment is limited to either intramuscular or subcutaneous administration, which compromises patient comfort (Tanko et al., 2004). A nasal spray that contains synthetic sCT is also available, but has limited use due to low bioavailability. The nasal cavity is a well known route of drug administration for the treatment of various diseases. Despite the extensive blood supply and the large surface area in the nasal cavity, the permeability of the nasal mucosa is normally low for polar molecules including low molecular weight drugs and especially large molecular weight peptides and proteins (Illum, 2007). Oral delivery is a comfortable and acceptable way to administer a large variety of drugs. The clinical development of orally active peptide drugs has been restricted by their unfavorable physiochemical properties, which limit their intestinal mucosal permeation (Thanou et al., 2001; Morishita and Peppas, 2006). In both the intestines and nasal cavity peptide drugs are also exposed to extensive digestion by proteases and proteinases (Thanou et al., 2001; Illum, 2007). Like other peptides, sCT is susceptible to cleavage by proteases and have poor absorption characteristics when administered orally. Intranasal delivery has been shown to be effective, but has a lower bioavailability than that of intramuscular or subcutaneous injections. This was attributed to the limited permeability of mucosal barriers. In this regard the absorption of sCT may be enhanced by using suitable drug carriers (Lee and Sinko, 2000; Torres-Lugo and Peppas, 2000).

There are many approaches to improve the absorption of peptides through both the oral and nasal delivery routes, including the use of liposomes (Chen et al., 2009), microspheres (Wang et al., 2006) and nanoparticles (Goycoolea et al., 2009). Various approaches have been followed to increase the nasal delivery of sCT. A chitosan-based (the glutamate salt) delivery system significantly increased the nasal delivery of sCT in sheep (Hinchcliffe et al.,

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2005). Liposomes have also been shown to be effective in increasing the nasal absorption of sCT in rabbits (Law et al., 2001) and more recently rats (Chen et al., 2009). Chitosan nanocapsules have been shown to be effective in enhancing the oral absorption of sCT in rats (Prego et al., 2006). These studies clearly emphasize the possibilities in enhancing the absorption of sCT in the presence of different absorption enhancers or in drug carrier systems in the nasal and oral route of administration. For a delivery system to successfully deliver a molecule into the systemic blood circulation it must carry a molecule through the mucous layer, over the glycocalix and across the epithelial layer into the lamina propria where there is sufficient blood perfusion for the drug to be absorbed (Dass and Choong, 2006; Thanou et al., 2001; Illum, 2007). In addition, it must protect the peptide drug molecule against enzymatic attack throughout the absorption process. A delivery system can, however, be designed to make use of some of the various existing epithelial transport mechanisms. These mechanisms include various transcellular transport carriers and paracellular diffusion. A drug carrier can for example be employed to target transport carrier proteins or to open tight junctions between the epithelial cells (Sarciaux et al., 1995; Lee, 1991; Hamman et al., 2005).

N-Trimethyl chitosan chloride (TMC) is a well described and studied polymer which opens tight junctions, improving paracellular diffusion of drug molecules. It is easy to incorporate TMC into drug formulations and absorption enhancement of peptide molecules can be achieved (Kotzé et al., 1998a,b, 1999; Van Der Lubben et al., 2001). The use of TMC is limited however to neutral and basic aqueous environments (Van der Merwe et al., 2004). Pheroid™ (referred to as Pheroid or Pheroids in further text) is a novel patented drug delivery system comprised of essential and plant fatty acids, which are emulsified in water that has been saturated with nitrous oxide. Pheroids has the capability to entrap, protect and deliver drug molecules across various biological membranes. Pheroids can be manipulated for various therapeutic applications and are able to entrap both hydrophilic and hydrophobic drugs. Furthermore, Pheroids can be prepared in different forms to manipulate its release characteristics (Saunders et al., 1999; Grobler et al., 2007). Lipid-based delivery systems have the ability to enhance gastrointestinal absorption of drugs via the selective lymphatic uptake of poorly bioavailable peptides and nasal absorption via delayed disappearance of peptides from the nasal cavity (Dass and Choong, 2006).

In this study the novel lipid-based colloidal Pheroid drug delivery system, was evaluated for its application in the nasal and intestinal delivery of sCT. The absorption enhancing characteristics of two different Pheroid formulations, namely Pheroid vesicles and Pheroid microsponges, were evaluated. This was compared with TMC, a well known absorption enhancer for macromolecular compounds across mucosal surfaces. Salmon calcitonin was administered intranasally and intestinally to rats in conjunction with Pheroid vesicles, Pheroid microsponges and TMC.

2. Materials and methods

2.1. Materials

Salmon calcitonin (with a nominal activity of 6000 IU/mg), 3431.9 Da was supplied by Sigma–Aldrich (St. Loius, MO). TMC with a degree of quaternisation of 48% and a molecular weight of 166 000 g/mole was prepared as previously described (Jonker et al., 2002). Vitamin F ethyl ester was obtained from Kurt Richter Pharma, Germany, Cremaphor® EL from BASF, Germany, *D*-α-tocopherol from Chempure, South Africa, Incromega 7010 and Incromega 3322 (used 1:1) from Croda Chemicals, South Africa. To determine plasma sCT concentrations a commercially available ultra sensitive RIA kit was used (DSL-3600, Diagnostics Systems Laboratories Inc., Texas, USA). Nile red was supplied by Sigma–Aldrich (St. Loius, MO).

2.2. Preparation of calcitonin TMC formulations

A stock solution of sCT in saline was prepared with a concentration of 1000 IU/ml. TMC polymer was carefully weighed and added to saline solution. This mixture was covered and stirred overnight to ensure that the TMC is properly dissolved and hydrated. The TMC solution was then added to an aliquot of the previously prepared sCT stock solution to yield solutions containing 100 IU/ml sCT and 0.5% (w/v) TMC for nasal delivery and 500 IU/ml sCT and 0.5% (w/v) TMC for intestinal delivery.

2.3. Preparation of sCT Pheroid formulations

Pheroid vesicles were prepared by mixing vitamin F ethyl ester (2.8% w/v), Cremaphor® EL (1% w/v) and *D*-α-tocopherol (0.2% w/v). This mixture was heated to 75 °C and constitutes the oil phase (4%) of the Pheroid vesicles. Purified water saturated with N_2O was heated and maintained at 75 °C. The previously heated oil mixture was then added to the heated N_2O saturated water (96%) to form the vesicles. The water-oil mixture was homogenised with a Heidolph Diax 600 homogeniser (Labotec, Johannesburg, South Africa) at 8000 rpm for 2 min. This homogenous oil-in-water emulsion (o/w) was then placed in amber glass bottles and shaken constantly until the mixture reached room temperature after which the bottles, now containing Pheroid vesicles, was placed in a refrigerator and kept at a temperature of 5 °C. Pheroid microsponges were prepared in exactly the same way as described above for the Pheroid vesicles, with the addition of Incromega 7010 and Incromega 3322 (0.25:0.25% w/v) to the oil phase before heating. The formulations containing sCT was prepared by adding a sufficient volume of freshly prepared Pheroid vesicles or microsponges to sCT aliquots (1:10). The formulations were slowly shaken for 30 min after which it was left overnight at 5 °C. These formulations contained Pheroids loaded with sCT at a concentration of 100 IU/ml.

2.4. Characterization of Pheroids

The mean particle size and particle size distribution of both the Pheroid vesicles and Pheroid microsponges after sCT entrapment were measured by light scattering with a particle size analyzer (Malvern Mastersizer, Malvern Instruments, United Kingdom). The instrument's laser was aligned with deionised water after which a diluted sample was added to the water. The samples were stirred continuously using a magnetic stirrer to obtain a homogenous dispersion of the vesicles or microsponges. The scattered intensity was registered at a scattering angle of 90° at 25 °C. The morphological features of Pheroid vesicles and Pheroid microsponges were assessed by both optical microscopic and confocal laser scanning microscopy (CLSM). The optical microscopy images were taken with an inverted microscope (Nikon Eclipse TE-3000). The CLSM images were taken with a Nikon D-Eclipse C1 confocal laser scanning microscope with a DXM 1200 digital camera with real time imaging and a medium (10 pm) pinhole. A 60× 1.40 ApoPlanar oil immersion objective was used. The microscope was equipped with a green krypton laser (wavelengths: excitation 488 nm, emission 515 nm) and a red helium neon laser (wavelengths: excitation 505 nm, emission 564 nm). Pheroids were labelled with the fluorophore Nile red (1 mg/ml). The latter has an emission wavelength of between 640 and 650 nm. Pheroids were placed on a microscope slide, covered with a glass coverslip and sealed with adhesive to prevent fluid loss.

2.5. In vivo administration of formulations

All surgical and experimental procedures were approved by the ethics committee of the North-West University. Male Sprague Dawley rats with body weights of approximately 300 g were fasted for 18 h, but water was supplied ad libitum. Six rats were used per treatment group. Anaesthesia was induced by liquid halothane (Fluothane®, Zebeca SA (Pty) Ltd., Woodmead, RSA), in medical oxygen. To maintain anaesthesia the rat was attached to a rubber jacket that fits securely over its head. The remaining end of the rubber jacket was connected to a two way selector which was connected to either one of two 5-l plastic bags. The bags contained either 2% (v/v) or 4% (v/v) halothane in oxygen. The *caroits communis* artery was cannulated for blood sampling and fluid replacement.

For nasal administration, the formulations were administrated at a dose of 10 IU/kg bodyweight. The required volume was delivered to the right nasal cavity using a micropipette. Care was taken not to damage the nasal epithelial layer in order to maintain its integrity ensuring accurate absorption profiles. For intestinal administration formulations were administrated at 500 IU/kg bodyweight injected directly into the jejunum of the rat. Control formulations consisted of saline solution containing either 100 IU/ml sCT or 500 IU/ml sCT. Intravenous formulations consisted of 100 IU/ml sCT in saline solution. There are some limitations in using rats to predict the oral bioavailability of peptide drugs in the presence of lipid-based delivery systems. Lipid digestion is a very important process for drug dissolution and absorption, but rats do not have a gall bladder which aids in lipid digestion (Han et al., 2009). TMC activity is limited to neutral and basic environments. The pH values of the stomach range between 2.5–3.7 in fasting state and between 6–7 in the duodenum, jejunum and ileum. The regional permeability of sCT is also highest in the ileum, followed by the jejunum and is lowest in the stomach (Shah and Khan, 2004). The study was therefore limited to intestinal administration of sCT Pheroid and TMC formulations.

Blood samples of ~1 ml were collected in Eppendorf® tubes (Merck, Darmstadt, Germany) at 0; 5; 10; 15; 30; 60; 120 and 180 min after both nasal and intestinal administration. Blood samples taken at each time interval were replaced with an equal volume of heparinized saline solution. The samples were centrifuged at 7000 rpm for 5 min and plasma carefully removed and kept at −70 °C until analysis could be performed.

2.6. Quantitative measurement of calcitonin in plasma

To determine the plasma sCT concentration a commercially available ultra sensitive RIA kit was used (DSL-3600, Diagnostics Systems Laboratories Inc., Texas, USA). The procedure follows the basic principle of radioimmunoassay where there is competition between a radioactive and a non-radioactive antigen for a fixed number of antibody sites. The amount of [I^{125}]-labelled bound sCT to the antibody is inversely proportional to the concentration of the unlabelled sCT present in the plasma sample. The separation of the free and bound antigen is achieved by using a double antibody system. The instructions provided by the manufacturer were followed exactly and the resulting vials were placed in a Gammatec™ II gamma counter (Canberra Company, South Africa) to determine the radioactivity and to calculate the plasma sCT concentration using a log-linear curve fit. All tubes were counted for 1 min.

2.7. Pharmacokinetic analysis

Plasma concentrations versus time data obtained for the different formulations in individual rats were analyzed by means of a one-compartment open model analysis method. The area under the curve (AUC) was calculated by the trapezoidal method. The maximum plasma concentrations (C_{max}) and the time to reach the maximum concentration (T_{max}) were determined from the pharmacokinetic profiles generated by plotting the concentrations of sCT in the different formulations against time. The absolute bioavailability was calculated by comparing the respective AUC's of the extravascular (EV) and intravenous (IV) administrations with the following equation:

$$\text{Absolute Bioavailability}\,(\%) = \left(\frac{\text{AUC}_{\text{EV}}}{\text{AUC}_{\text{IV}}}\right) \times \left(\frac{\text{Dose}_{\text{IV}}}{\text{Dose}_{\text{EV}}}\right) \times 100$$

Table 1
Particle size analysis of Pheroid vesicles and Pheroid microsponges (volume weighted data).

Formulation	$D_1 = 0.1$[a]	$D_2 = 0.5$[b]	$D_3 = 0.9$[c]
Pheroid vesicles	0.5 μm	1.1 μm	3.3 μm
Pheroid microsponges	0.6 μm	1.6 μm	3.8 μm

[a] 10% of the particles present in the mixture are below this size.
[b] This is the median particle size of the sample.
[c] 90% of the particles present in the mixture are below this size.

2.8. Statistical analysis

All values are expressed as the mean ± standard deviation (SD). To determine statistical differences the data were analyzed by one-way ANOVA using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). A p value of less than 0.05 was termed significant.

3. Results

3.1. Characterization of Pheroid formulations

The particle sizes of the Pheroid formulations are summarized in Table 1. The particle size distribution curve showed a narrow size distribution for Pheroid vesicles with 80% of the vesicles with sizes between 0.5 μm and 3.3 μm, with a median size of 1.1 μm. Pheroid microsponges were slightly larger with 80% of the microsponges between 0.6 μm and 3.8 μm, with a median size of 1.6 μm. Fig. 1 shows CLSM and optical microscopy micrographs of the Pheroid formulations. The images clearly show the spherical microstructure of the Pheroids. The Pheroid vesicles and Pheroid microsponges readily associated with Nile red and form a striking red contrast with the background. The highly red fluorescent lipid bilayer can clearly be seen, with the inner aqueous core represented by the dark (black), non-fluorescent part of the structure. Pheroid vesicles (Fig. 1A) are uniform in shape with a vesicular structure, whereas the porous nature of Pheroid microsponges are visible (Fig. 1B). No differences in the particle size and morphological features were seen before and after entrapment of sCT (data not shown).

3.2. Nasal absorption of sCT

Fig. 2 shows the plasma sCT concentration–time curve after intravenous injection of sCT. Fig. 3 shows the mean plasma sCT concentration (pg/ml) at various times (min) after nasal administration of the different formulations. Negligible absorption was observed for the control formulation. Both the TMC and Pheroid formulations were able to increase the plasma sCT concentrations after 5 min, reaching maximum concentrations around 15 min, where after concentrations started to decrease towards initial plasma concentration values. The Pheroid vesicle formulation increased the absorption of sCT significantly at 5, 10, 15 and 30 min ($p < 0.05$ when compared to the control). Pheroid microsponges increased sCT absorption significantly at 5, 10 and 15 min. The TMC formulation gave significantly higher absorption than the control and

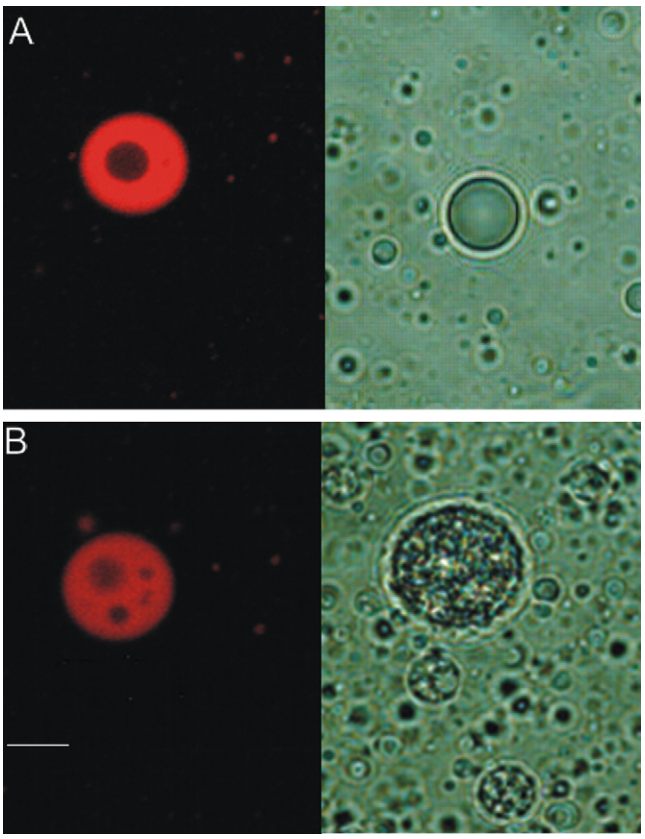

Fig. 1. Confocal scanning microscopy and optical images showing different Pheroid formulations. The Pheroids are visualized by labeling with Nile red, which associated with lipid molecules and emits red fluorescence. (A) Pheroid vesicles and (B) Pheroid microsponges. The scale bars represents 1 μm.

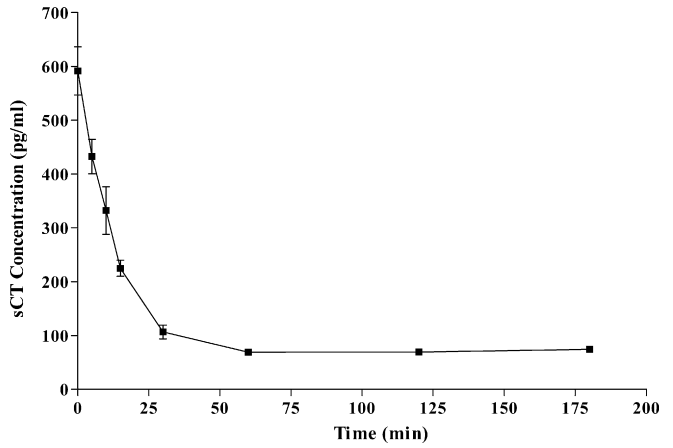

Fig. 2. Plasma sCT concentration–time curve after intravenous administration of sCT at 10 IU/kg bodyweight. Each data point represents the mean ± SD, $n = 6$.

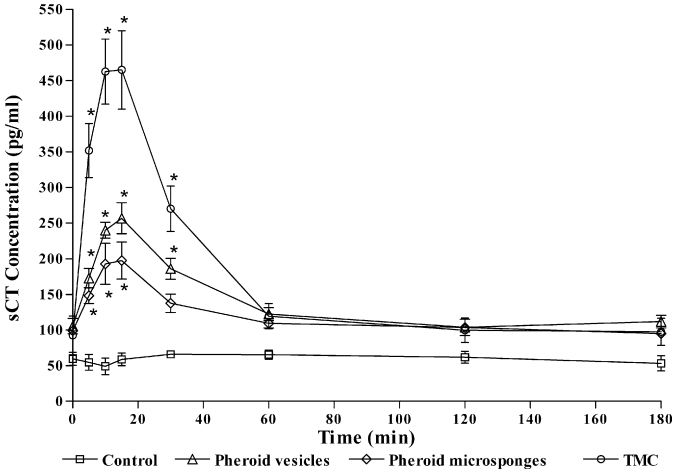

Fig. 3. Plasma sCT concentrations after nasal administration of sCT (10 IU/kg bodyweight). Each data point represents the mean ± SD, $n = 6$, * indicate significant differences compared to the control ($p < 0.05$).

both Pheroid formulations at 5, 10, 15 and 30 min ($p < 0.05$). In Table 2 the pharmacokinetic parameters, after nasal administration of sCT, are summarized. The formulation containing TMC showed the highest C_{max} with a value of 478.5 ± 6.1 pg/ml and gave maximum absorption levels after an average time of 12.5 ± 4.7 min. The C_{max} value calculated for the Pheroid vesicle formulation was 262.6 ± 17.1 pg/ml and it was reached after 14.7 ± 1.7 min. This was significantly lower compared to the absorption profile obtained with TMC, but still higher than that of the Pheroid microsponge formulation with an average C_{max} of 202.7 ± 28.6 after 11.7 ± 1.7 min. However, compared to the control group, both the Pheroid vesicle and Pheroid microsponge formulations showed significant increases in AUC-values as well as in C_{max} values. A clear distinction in terms of relative bioavailability was observed between the formulations and the bioavailability of sCT after administration of TMC was approximately 1.5-fold higher than that of the Pheroid vesicle and Pheroid microsponge formulations and nearly 3 times

Table 2
Pharmacokinetic parameters in Sprague Dawley rats after intranasal administration of 10 IU/kg bodyweight sCT in various formulations ($n = 6$).

Formulation	AUC	C_{max} (pg/ml)	T_{max} (min)	Absolute bioavailability (%)
IV	19177 ± 597.8	591.78 ± 45.1	0	100
Control	10959 ± 1356.4	72.57 ± 6.1	34.2 ± 18.4	56.42
Pheroid vesicles	24048 ± 1739.5*	262.64 ± 17.1*	14.1 ± 1.7	123.81
Pheroid microsponges	20962 ± 1191.6*	202.66 ± 28.6*	11.7 ± 1.7	107.92
TMC	28509 ± 1356.4*	478.5 ± 6.1*	12.5 ± 4.8	146.77

* Significant differences compared to the control ($p < 0.05$).

Table 3
Pharmacokinetic parameters in Sprague Dawley rats after intestinal administration of 500 IU/kg bodyweight sCT in various formulations ($n = 6$).

Formulation	AUC	C_{max} (pg/ml)	T_{max} (min)	Absolute bioavailability (%)
Control	32380 ± 1061.4	249.1 ± 21.5	11.6 ± 1.4	3.33
Pheroid vesicles	37632 ± 1239.8	386.2 ± 45.5	5.1 ± 1.7	3.87
Pheroid microsponges	40600 ± 1491.8*	432.1 ± 18.9*	10.7 ± 2.7	4.18
TMC	633353 ± 1186.7*	738.9 ± 277.1*	14.5 ± 2.8	6.52

* Significant differences compared to the control ($p < 0.05$).

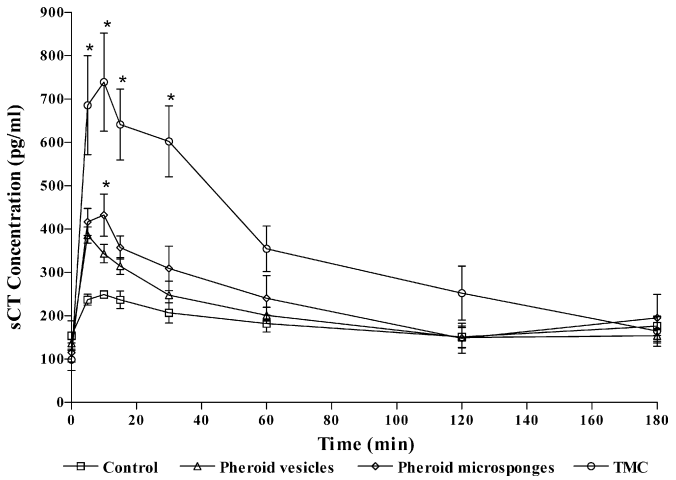

Fig. 4. Plasma sCT concentrations after intestinal administration of sCT (500 IU/kg bodyweight). Each data point represents the mean ± SD, $n = 6$, * indicate significant differences compared to the control ($p < 0.05$).

higher than that of the control formulation. No significant differences in bioavailability were seen between the Pheroid vesicle and Pheroid microsponge formulations.

3.3. Intestinal absorption of sCT

Fig. 4 shows the effect of the different formulations on sCT absorption when administrated intestinally. Slightly better absorption of sCT in the control formulation was obtained when compared to nasal administration. In the control formulation the plasma sCT concentration reached a peak after 10 min and similar to nasal absorption reached initial sCT levels again after 60 min. The Pheroid microsponge formulation increased the absorption of sCT significantly at 10 min (when compared to the control $p < 0.05$). Pheroid vesicles gave slightly better absorption compared to the control, but these differences were not significant. As with nasal administration, the formulation with TMC resulted in major sCT absorption with significant differences at 5, 10, 15 and 30 min. Even after 120 min the concentration was still 252.27 pg/ml after which it gradually decreased to the initial value obtained before sCT administration. Table 3 summarizes the pharmacokinetic parameters after intestinal administration of sCT. Pheroid microsponges and TMC had significantly higher AUC and C_{max} values when compared to the control. Regarding the high dose administered orally, the relative bioavailability of sCT were low compared to nasal administration. The bioavailability of sCT in combination with TMC increased 2-fold compared to the control, whereas slight differences in bioavailability were seen with the Pheroid formulations.

4. Discussion

The Pheroid drug delivery system is a colloidal system with unique lipid-based structures that differ significantly from liposomes. The dispersed structures can be manipulated in terms of morphology or size. In this study two type of Pheroid formulations were formulated and characterized with CLSM. Pheroid vesicles and Pheroid microsponges readily associate with Nile red, a fluorescent lipophilic dye with red emission. Optical sectioning with laser allows scanning through a structure without physical interference. Pheroid vesicles are small (ca. 1 μm) liposome-like structures with a bilayer membrane and hydrophilic core. However, the lipid bilayer contains no phospholipids or cholesterol. Pheroid microsponges are slightly larger than Pheroid vesicles with a mean diameter of 1.6 μm and have a porous nature. The central hydrophilic aqueous space of both vesicles and microsponges can be used for entrapment of hydrophilic compounds, whereas hydrophobic compounds can be entrapped in the elastic lipid membrane. As peptides are hydrophilic they would associate with the inner aqueous phase of the Pheroid vesicles or Pheroid microsponges. Pheroids are formed by a partial self-assembly process similar to that of certain types of micro-emulsions and no lyophilization or hydration of the lipid components are necessary. The nitrous oxide most likely contributes to the miscibility and self-assembly of the Pheroids and may play an important role in the stability of the system (Grobler et al., 2007). These factors are currently under investigation.

The absorption enhancing effect of Pheroid vesicles and Pheroid microsponges were evaluated by intranasal and intestinal administration of sCT to rats with assessment of sCT plasma levels. There were no apparent adverse systemic events in the rats following nasal or intestinal sCT administration and the Pheroid formulations appeared to be well tolerated. The mean plasma concentration–time profiles of nasally administered sCT were similar for Pheroid vesicles and Pheroid microsponges, albeit higher for Pheroid vesicles. These profiles were characterized by an early sCT peak, indicative of rapid absorption and distribution of the drug, followed by a slower decline due to elimination (Fig. 3). Absolute bioavailability of nasally administered Pheroid formulations resulted in values above 100%. This could possibly be attributed to the fast clearance of the IV formulation from the blood, in contrast to the delayed elimination with the Pheroid vesicles and Pheroid microsponges (Figs. 2 and 3). Intestinal administration of sCT in Pheroid vesicles resulted in an earlier peak compared to Pheroid microsponges, but in contrast to nasal administration gave lower plasma sCT levels.

One of the mechanisms of action of Pheroids was proposed to be that of protein-mediated transfer by intracellular fatty acid-binding proteins (active transport) (Grobler et al., 2007). On the other hand epithelial cell membranes consist of a phospholipid bilayer with its lipophilic components arranged to the inside of the membrane, much like the proposed structure of Pheroids. With these similar properties in mind there is also the possibility of transcellular passive diffusion of drug loaded Pheroid vesicles across cell membranes. The differences in absorption between the Pheroid vesicles and Pheroid microsponges can possibly be attributed to the differences in the chemical composition of the membranes. During the preparation of Pheroid microsponges the addition of Incromega to the formulation has such a profound effect on the formation of the vesicles that changes in vesicle size and in the visible structure of the vesicles can be seen. Pheroid microsponges can therefore be regarded as a different delivery system. Although there are many shared properties, the difference in size alone can influence passive diffusion across membranes (Pouton, 2000); the difference in membrane structure can also influence receptor mediated transport if present (Chakraborty et al., 2009). All these factors could therefore be possible reasons for the slight difference in the absorption enhancing properties of the Pheroid vesicle and Pheroid microsponge formulations.

TMC is a well known partially quaternised derivative of chitosan with superior solubility that can increase the absorption of hydrophilic and macromolecular compounds by paracellular diffusion (Kotzé et al., 1998a). The results of this study showed that it is possible to improve the absorption of sCT significantly after nasal and intestinal administration in rats when administered with TMC. The plasma concentration–time curves of sCT after nasal and intestinal administration with TMC was significantly higher compared to the control, Pheroid vesicle and Pheroid microsponge formulations. The absorption enhancing effect of TMC can be attributed to interactions of the fixed positively charged quaternary amino groups on the C-2 position of TMC with the

negatively charged anionic components on the cell surfaces and tight junctions (Kotzé et al., 1998a,b). TMC is good polymeric candidate for peptide drug delivery due to its good water solubility and mucoadhesive properties. It was shown previously that co-administration of TMC polymers increased the absorption of hydrophilic macromolecules, such as peptide drugs (Kotzé et al., 1997; Van der Merwe et al., 2004). It was also shown that the optimum degree of quaternisation for TMC was 48% and that no further significant increases in absorption were obtained with TMC with higher quaternisation values. This was attributed to a charge threshold value which induced significant interactions to open the tight junctions between adjacent epithelial cells (Jonker et al., 2002; Hamman et al., 2002).

5. Conclusion

Considerable increases in the nasal absorption of sCT were obtained after formulation in TMC polymer solution and in both Pheroid vesicle and Pheroid microsponge formulations although the TMC formulation was more effective in enhancing the absorption of sCT across the nasal epithelium, compared to the Pheroid formulations. Similar results were obtained with intestinal administration of sCT, although not as profound as with nasal administration. TMC is an effective permeation enhancer especially for the mucosal delivery of protein and peptides, but is limited by its solubility in neutral and basic environments. As a tight junction modulator there is still a possibility that enhanced transport of other molecules including xenobiotics or pathogens, may take place. TMC is also a derivative of chitosan which is not listed on the FDA inactive ingredient database. Pheroids consist of essential fatty acids natural to the body. The Pheroid drug delivery system as a lipid-based drug delivery system has the advantage of delaying nasal clearance of drugs and increasing gastrointestinal absorption of drugs via selective lymphatic uptake. Due to different absorption mechanisms all these formulations show promise for further investigation and novel dosage form development. TMC and Pheroid technology may provide a practical means for non-injectable delivery of sCT and potentially other therapeutic peptide and protein molecules.

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