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International Journal of Pharmaceutics 269 (2004) 303–310
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

In vitro evaluation of intestinal absorption of desmopressin using drug-delivery systems based on superporous hydrogels

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Received 2 June 2003; received in revised form 1 August 2003; accepted 6 September 2003

Abstract

The aim of this study was to investigate and modify the potential of drug-delivery systems based on superporous hydrogel (SPH) for improving the intestinal transport of the peptide drug desmopressin in vitro. The swelling properties and mechanical strength of SPHs were studied. The release profile of desmopressin was investigated by changing the composition of excipients in the formulations. Subsequently, the ability of the SPH-based drug-delivery systems to enhance the transport of desmopressin across porcine intestine was performed in vitro. The swelling properties and mechanical strength of SPHs were affected by the addition of the disintregrant AcDiSol®. This disintregrant reduced the swelling ratio to 10% and the time to 80% swelling was retarded by 3–5 min in comparison to the negative control. AcDiSol® increased the mechanical strength, according to the increasing of penetration pressure value, the pressure that the punch can penetrate the gel, of the SPHs. The transport of desmopressin across the intestinal mucosa in vitro was enhanced four- and six-fold by applying SPH, with AcDiSol®, in the absence and presence of the additional absorption enhancer trimethyl chitosan chloride, respectively, in comparison to the negative control. It is concluded that drug-delivery systems based on SPHs are promising for enhancing the intestinal absorption of desmopressin.

Keywords: Desmopressin; Superporous hydrogels; Trimethyl chitosan chloride; Intestinal absorption in vitro; Absorption enhancers

1. Introduction

Normally poor bioavailability of peptides and proteins applied by the oral and non-oral mucosal routes is a result of the interplay of poor permeability characteristics, instability towards proteolytic enzymes, cell metabolism and non-enzymatic clearance mechanisms such as the first-pass effect and excretion in the bile (Fix, 1996). Various types of polymers have shown to enhance the oral bioavailability of peptide and protein drugs (Junginger and Verhoef, 1998). Beyond these biodegradable polymers as absorption enhancer, different peptide analogues have been developed by chemical modification of the peptide structure to prevent proteolytic degradation and increase the bioavailability of peptide drugs (Lundin et al., 1991).

Several approaches to enhance the peroral delivery of peptides and proteins are currently under investigation. One of them is a peroral drug-delivery system based on superporous hydrogels. In recent years, such

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doi:10.1016/j.ijpharm.2003.09.022

systems consisting of superporous hydrogel (SPH) and superporous hydrogel composite (SPHC) polymers were developed. SPH and SPHC polymers are a new generation of hydrogels (Chen and Park, 2000). When these polymers are delivered into the intestine, they are able to mechanically stick for a certain period of time at the gut wall, sucking up deleterious luminal fluids and opening the tight junctions before finally releasing the peptide drug. (Dorkoosh et al., 2002a). After having released the peptide in a time-controlled manner, the SPH(C) polymers become superhydrated and are easily broken down by the peristaltic force of the gut and subsequently excreted as fine particles. This action principle was verified in an in vivo study in pigs and octreotide as peptide drug: using various designs of SPH/SPHC delivery systems applied in enteric-coated capsules, absolute bioavailabilities up to 16% were obtained (Dorkoosh et al., 2002b). A recent γ-scintigraphic study in human volunteers has shown that SPH(C) polymers enhance the residence time of such delivery systems in the intestinal tract for at least 1 hour due to their chemical fixation properties, and that these systems are safe in use (Dorkoosh et al., 2003)

The biodegradable polymer *N*-trimethyl chitosan chloride (TMC) is a partially quaternised derivative of chitosan and is well-soluble over a wide range of pH values. TMC has proven to be a potent intestinal absorption enhancer for the peptide drugs buserelin and octreotide. This polymer is able to open tight junctions, which seal the paracellular pathways, thereby facilitating the paracellular diffusion of the peptide drugs Thanou et al. (2000). The process has the additional advantage of being reversible by removing the polymer, leading to the resealing of the tight junctions. It has been shown that co-administration of octreotide with 5 and 10% (w/v) solutions of TMC at a pH of 7.4 in the jejunum of pigs resulted in a 7.7- and 14.5-fold increase of ocreotide absorption, respectively (Thanou et al., 2001).

Desmopressin [1-(mercaptopropionic acid)-8-D-arginine vasopressin; DDAVP] is a synthetic analogue of the antidiuretic hormone vasopressin. The parent molecule, vasopressin, was altered in two positions: removal of the N-terminal amino group and substitution of L-arginine with D-arginine, which lead to a significantly prolonged duration of the antidiuretic activity and a reduced blood pressure effect. Desmopressin is active in the treatment of central diabetes insipidus and nocturnal enuresis (Vilhardt, 1990). It is used in the management of primary nocturnal enuresis in children and adults as well as the treatment of central diabetes insipidus. Currently, DDAVP is administered orally as a tablet formulation (Minirin, Ferring Pharmaceuticals), but the bioavailability is lower than 1% (Vilhardt and Lundin, 1986).

In this study, optimized superporous hydrogels in combination with highly quarternized TMC were evaluated for their potential to enhance the transport of a peptide drug (desmopressin) across porcine intestine in vitro.

2. Material and methods

2.1. Materials

Desmopressin acetate was obtained from Ferring Pharmaceuticals (Malmö, Sweden). Acrylic acid, acrylamide, 3-sulfopropyl acrylate potassium, *N*,*N*′-methylenebisacrylamide, Pluronic F127, ammonium persulfate, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine and sodium bicarbonate were purchase from Sigma (Zwijndrecht, The Netherlands). AcDiSol® was from FMC Corp. (Philadelphia, PA). Chitosan (97% deacetylated, viscosity 552 mPa s) was obtained from Primex Biochemicals (Haugesund, Norway). *N*-Methylpyrrolidinone and methyl iodide were obtained from Acros Organics (Geel, Belgium). The water used was filtered by a Milli-Q UF plus ultrapure water system from Millipore (Etten-Leur, The Netherlands). All the other chemicals were commercially available and were used as received.

2.2. Methods

2.2.1. Synthesis of superporous hydrogels

Superporous hydrogels were synthesized as firstly described by Chen and Park (2000) and adapted for intestinal purposes by Dorkoosh et al., 2002. Briefly, the following substances were added subsequently into a glass test tube (inner diameter of 15 mm, and height of 93 mm) at ambient temperature: 2 ml mixture of 50% acrylamide (AM) and 50% 3-sulfopropyl acrylate potassium (SPAK) (4:3, v/v); 450 μl of 2.5% *N*,*N*′-methylenebisacrylamide (BIS); 100 μl of 10%

Pluronic F127 (P127); 60 μl of 50% acrylic acid (AA); 45 μl of 20% ammonium persulfate (APS). The test tube was shaken to mix the solution after each ingredient was added. The AcDiSol® powder was added to the test tube and mixed vigorously. Then, 45 μl of 20% *N*,*N*,*N*′,*N*′-tetramethyethylenediamine (TEMED) was added to the mixture and the test tube was shaken. Finally, 120 mg of sodium bicarbonate ($NaHCO_3$) powder was added and the mixture was immediately stirred vigorously using a spatula for 15 s. The polymerization was allowed to continue at room temperature for approximately 30 min. The superporous hydrogels were retrieved from the test tube, and extensively washed with distilled water to remove any residual monomer. The hydrogels were dehydrated with alcohol and were dried in an oven at 60 °C for 1 day. The amount of AcDiSol®, the composite material, was varied from 0 to 200 mg (0–14.7%, w/w).

2.2.2. Swelling studies

The dry samples were allowed to hydrate in pancreatin-free simulated intestinal fluid (SIF; pH 6.8) at room temperature. The SIF was prepared according to USP XXIII, except that pancreatin was not added. The weight of the hydrating samples was measured at timed intervals, following removal of excess swelled water by gentle blotting. Some superporous hydrogels swelled to the extent that they became too fragile to handle. Those were placed on a sieve weighing boat (Dorkoosh et al., 2000). This weighing boat containing the superporous hydrogel was immersed in the water for swelling. The weighing boat was taken out to drain free water from the sieve and a paper towel was used to remove excess water from underneath the sieve. Then the weight of the swollen superporous hydrogels was measured by subtracting the boat weight from the total weight. This method avoided the direct handling of the fragile superporous hydrogels (Dorkoosh et al., 2000). The swelling ratio (Q) is defined as:

$$Q = \frac{W_s - W_d}{W_d} \quad (1)$$

where W_s is the weight of the swollen superporous hydrogel and W_d is the weight of the dried superporous hydrogel.

2.2.3. Mechanical strength study

The mechanical strength of the superporous hydrogels was tested using a bench comparator as described by Chen et al. (2000). After being swollen in SIF for approximately 1 h, a superporous hydrogel sample was placed longitudinally under the lower touch of the comparator in a container filled with SIF. The contact area of lower touch was about 50 mm^2. Weight was applied to the upper touch of the comparator until the structure fractured and could not support more weight. The mass, m, was converted to pressure (P) using equation:

$$P = \frac{mg}{A} \quad (2)$$

where g is the acceleration due to the gravity and A is the contact area of the lower punch. The strength that the gel could not withstand was called "penetration pressure (PP)," because the punch penetrated into the superporous hydrogels. The penetration pressure appeared to be more accurate than the ultimate compression pressure (UCP) which was described before (Chen et al., 2000; Shalaby and Park, 1990). UCP is defined as the point that the superporous hydrogel started cracking, but this point can not easily be determined due to the physical characteristics of the superporous hydrogels. Moreover, the investigated hydrogels were not fully immersed in the swelling medium during examination.

2.2.4. Synthesis of *N*,*N*,*N*-trimethyl chitosan chloride

N,*N*,*N*-Trimethyl chitosan chloride (TMC) was synthesized as previous described with some modification (Sieval et al., 1998). Briefly, chitosan was mixed with methyl iodide in an alkaline solution of N-methylpyrrolidinone at 60 °C for 60 min. This obtained product underwent a second step of reductive methylation for 60 min, subsequently with an addition step for 60 min. The product trimethyl chitosan iodide was precipitated with ethanol and diethyl ether. The purification step of the product included the exchange of the counter ion iodide with chloride in an aqueous NaCl solution. The polymer was precipitated from solution using ethanol and diethyl ether. Finally, the product was dissolved in 40 ml of water to remove the remaining NaCl and precipitated from solution with ethanol and diethyl ether. The product was dried in a vacuum chamber for at least

12 h. TMC polymer was measured for its degree of quaternization by ^{1}H NMR, using a 400 MHz spectrometer (Bruker, Switzerland), which was found to be 77%.

2.2.5. In vitro dissolution test

The tablets containing 5 mg desmopressin, 10–15 mg TMC, 5–7.5 mg AcDiSol® and lactose monohydrate qs to 50 mg, were compressed by a FTIR compress machine to tablets with a diameter of 3 mm. Two tablets of desmopressin were attached to the superporous hydrogel with a biodegradable glue (Dorkoosh et al., 2002b). The in vitro dissolution tests were investigated by using the USP XXIII paddle apparatus. The rotation speed was 100 rpm and the dissolution medium was 100 ml SIF, pH 6.8 at 37 ± 1 °C. Volumes of 2 ml were withdrawn at predetermined interval times from the dissolution medium and were replaced with fresh medium. The samples were analysed by HPLC as described below.

2.2.6. Transport across porcine intestine in vitro

Freshly isolated pieces of porcine intestine (jejunum) were obtained from a local slaughterhouse on the day of the experiments, transported to the laboratory in an ice-cooled box and were used within 1 h. The intestine was washed with physiological saline (0.9% NaCl) to remove food residues. The intestine was cut into pieces of approximately 15 cm each. The pieces of intestine were tied up at one end with a silk thread. After placing the superporous hydrogel with desmopressin in the intestine, 20 ml of SIF containing 0.33% glucose (pH 6.8) was added into the intestine not only to allow the polymer to swell, but also to allow desmopressin to release. The other open end of intestine was also tied with a silk thread to form a sac (Dorkoosh et al., 2002a). In case of the negative control, 20 ml solution of desmopressin (1 mg/ml) was placed in the intestinal sac. The filled sacs were immersed in the container filled with 200 ml of oxygenated SIF containing 0.33% glucose (pH 6.8). This solution was mixed very smoothly with a turning paddle (40 rpm) and the container was placed in a water bath at 37 ± 1 °C. Samples of 2 ml were withdrawn from the serosal side at predetermined interval times and analysed by HPLC as described below. All experiments were performed four to five times.

2.2.7. Analytical methods

Samples were measured on desmopressin by reverse phase-HPLC. Isocratic elution was carried out with 25% acetonitrile and 75% trifluoroacetic acid 0.1% (w/v; Law et al., 2001). The detection wavelength was 220 nm. The column used was Chromspher 5 C18, 250 mm × 4.6 mm (Chrompack, Middelburg, The Netherlands) equipped with a Chromsphere 5 C18, 10 mm × 4.6 mm precolumn. The flow rate was 1 ml/min and the temperature was ambient. The injection volume was set at 50 μl. Retention time of desmopressin was 7–8 min, and the detection limit was 0.08 μg/ml.

3. Results and discussion

3.1. Swelling studies

The effect of various amounts of AcDiSol® added into the superporous hydrogel was examined. AcDiSol® affected both the swelling ratio and swelling kinetics of the hydrogels. The swelling ratios (Q) of the superporous hydrogels are shown in Table 1. By the addition of AcDiSol®, the swelling ratio of the superporous hydrogels was reduced about 10%, but the observed differences were not significant.

The swelling kinetics of superporous hydrogels are depicted in Fig. 1. The initial swelling rate of the hydrogels without AcDiSol® was slower than that with AcDiSol®. The time to reach 80% swelling ($S_{80\%}$) was 10.3 ± 2.2 min for superporous hydrogel without AcDiSol®. For superporous hydrogel with AcDiSol® in amounts of 50, 100, 150 and 200 mg, $S_{80\%}$ values were 7.1 ± 4.3, 4.5 ± 1.4, 3.4 ± 1.1 and 2.4 ± 0.4 min, respectively.

Table 1
Swelling ratio of superporous hydrogels with various amounts of AcDiSol®

AcDiSol®		Swelling ratio (Q)	
Amount added (mg)	% (w/w)	Mean	S.D. (n)
0	0	50.87	1.64 (4)
50	4.13	45.62	3.31 (4)
100	7.93	43.83	2.50 (4)
150	11.45	42.82	1.75 (4)
200	14.70	42.82	1.85 (4)

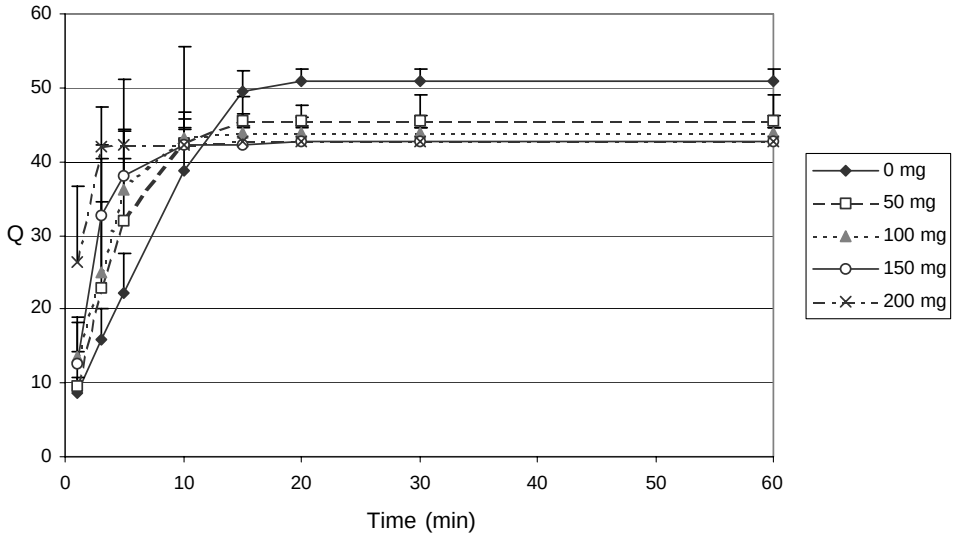

Fig. 1. The effect of AcDiSol® on the swelling kinetics of superporous hydrogels. Data are expressed as the mean ± S.D. ($n = 4$).

3.2. Mechanical strength study

From the penetration pressure studies, it was found that AcDiSol® slightly increased the penetration pressure value of the superporous hydrogels. Fig. 2 shows the relationship between the PP and the amount of AcDiSol® added. The mechanical strength of the superporous hydrogels was significantly increased by the addition of 150 mg (11.4%, w/w) of AcDiSol®, whereas the mechanical strength of the hydrogels with 150 and 200 mg (14.7%, w/w) of AcDiSol® was not significantly different ($P > 0.05$). The superporous hydrogel with 150 mg of AcDiSol® was selected for further studies.

AcDiSol® is a commonly used disintegrant in tablets and capsules to promote their fast disintegration. It is a hydrophilic insoluble cellulose and exists as long fibres with a hollow lumen. In contact with

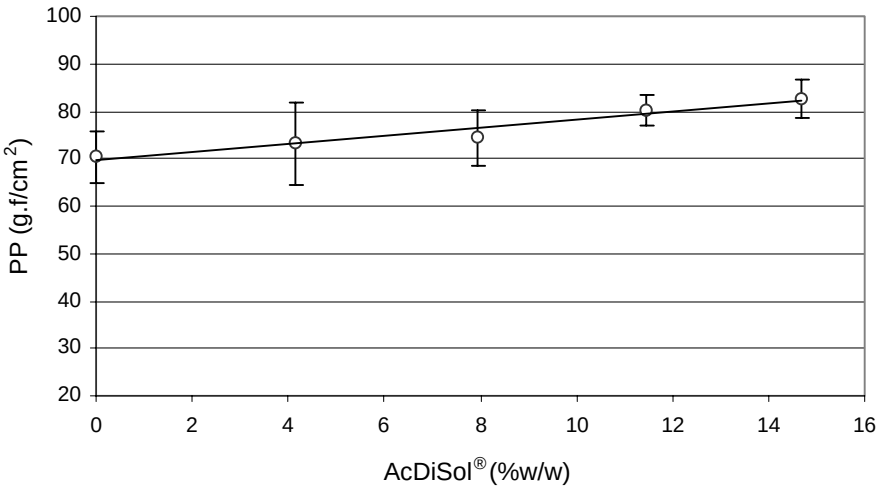

Fig. 2. Mechanical strength of superporous hydrogels with various amounts of AcDiSol®. Data are expressed as the mean ± S.D. ($n = 4$).

aqueous media, AcDiSol® absorbs water and then expands (Shangraw et al., 1981; Chen and Park, 2000). The swelling action of the AcDiSol® can expand the capillary channels in the hydrogel. The AcDiSol® also provides intrafibrous capillary channels in its hollow lumens (Chen et al., 2000). These effects cause the reduction of the swelling time of the hydrogel.

It is possible that AcDiSol® improved the structural integrity of the superporous hydrogel by entanglement of the polymer chains with AcDiSol® fibres. Thus, the overall cross-linking density increased, thereby decreasing the swelling ratio.

The presence of AcDiSol® increased the viscosity of the mixture, resulting in the more stable foam, which allows for easier control of the synthesis process. However, a too high amount of AcDiSol® will affect the mixing process, due to the increased viscosity of the mixture.

3.3. In vitro dissolution test

The results of desmopressin release from the hydrogels are depicted in Fig. 3. It was shown that the dissolution rate of desmopressin from tablets containing AcDiSol® were faster than the dissolution rate from tablets without AcDiSol®. The time to 80% dissolution ($T_{80\%}$) was about 25 min for the tablets containing 20% TMC without AcDiSol®. The $T_{80\%}$ values of tablets containing 20% TMC and 10% AcDiSol® was about 10 min, and that of tablets containing 30% TMC and 15% AcDiSol® was about 20 min. This can be attributed to the properties of TMC. TMC is a polymer which forms a gel-like micro-environment when it is dissolved in the dissolution medium. Increasing the amount of AcDiSol® from 10 to 15% can not overcome the effect of increasing the amount of TMC from 20 to 30%. Both tablets containing 20% TMC and 10% AcDiSol® and tablets containing 30% TMC and 15% AcDiSol® have been chosen to the study of desmopressin transport across porcine intestine in vitro.

3.4. Transport across porcine intestine in vitro

The results of desmopressin transport across porcine intestine in vitro are shown in Fig. 4. In the absence of any polymer (control), the amount of transported desmopressin was $0.44 \pm 0.18\%$ of the applied dose after 240 min. When desmopressin was applied in the superporous hydrogel dosage form into the intestine, it was transported up to $1.77 \pm 0.72\%$. In case, the peptide drug was applied with the hydrogel and TMC in amounts of 20 and 30% by weight, the transported percentage was $2.77 \pm 0.34\%$ and $2.82 \pm 0.76\%$ of the applied dose, respectively. Both superporous hydrogels

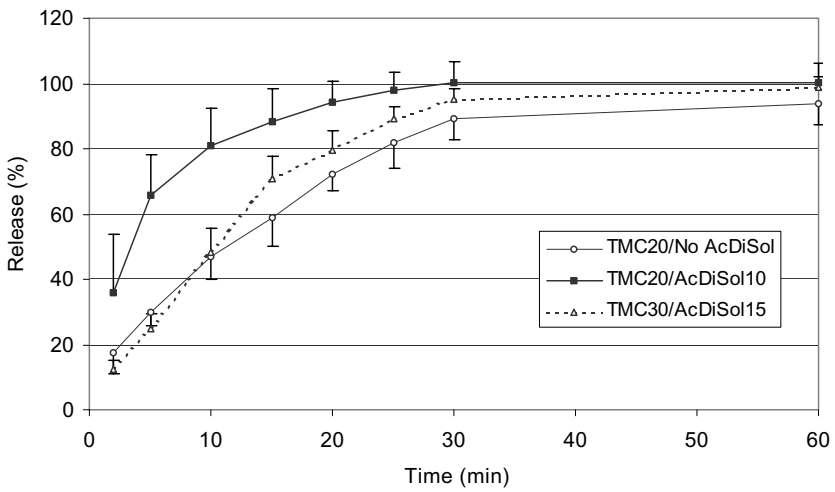

Fig. 3. Release of desmopressin from drug-delivery systems based on superporous hydrogels. Data are expressed as the mean ± S.D. TMC20/No AcDiSol = DDAVP 10%, TMC 20%, lactose 70% by weight ($n = 3$); TMC20/AcDiSol10 = DDAVP 10%, TMC 20%, AcDiSol® 10%, lactose 60% by weight ($n = 3$); TMC30/AcDiSol15 = DDAVP 10%, TMC 30%, AcDiSol® 15%, lactose 45% by weight ($n = 4$).

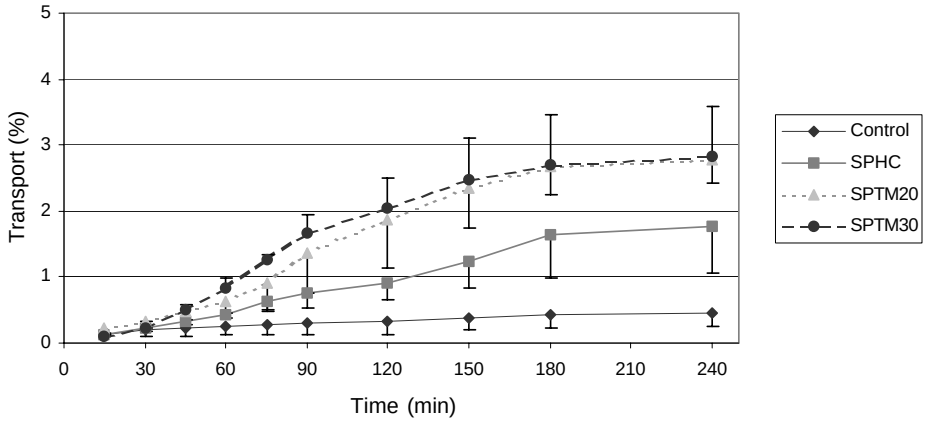

Fig. 4. Transport of desmopressin across porcine intestine in vitro. Data are expressed as the mean ± S.D. Control = 20 ml solution of 1mg/ml desmopressin ($n = 5$); SPHC = 2 tablets of desmopressin (5 mg/tablet) attached to SPH polymer ($n = 4$); SPTM20 = 2 tablets of desmopressin (5 mg/tablet) and TMC (10 mg/tablet) attached to SPH polymer ($n = 4$); SPTM30 = 2 tablets of desmopressin (5 mg/tablet) and TMC (15 mg/tablet) attached to SPH polymer ($n = 3$).

without TMC and hydrogels with TMC (20 and 30%) improved the cumulative transport of desmopressin 4.0-, 6.3- and 6.4-fold, respectively, compared to the control. The potential of superporous hydrogels to enhance the transport of desmopressin is caused by opening of the tight junctions due to mechanical pressure (Dorkoosh et al., 2002a). On the other hand, the accumulation of desmopressin at the mucosal membranes will force the diffusion across the opened junctions. In case of TMC application with the superporous hydrogel, TMC additionally enhances the paracellular transport of desmopressin. The hydrogels with 20 and 30% TMC resulted in a 1.5-fold enhancement of desmopressin transport compared to superporous hydrogels without TMC. Because most of the tight junctions were already opened by the effect of mechanical pressure from the superporous hydrogels, the effect of the different amounts of TMC (20 and 30%) was not significantly different ($P > 0.05$).

4. Conclusion

The present studies showed that the disintegrant AcDiSol® can optimize the properties of the superporous hydrogels as peroral dosage forms for peptide drugs. These delivery systems with and without TMC as additional absorption enhancer improved the transport of desmopressin across porcine intestinal membranes in vitro by opening the tight junctions and increasing the drug concentration at the surface of the mucosal epithelium. The potential of these systems for oral delivery of desmopressin is currently investigated in pigs in vivo.

Acknowledgements

The authors would like to thank the Royal Thai Government and Ministry of University Affairs for financial support of A.P. They are also grateful to Slagerij Van den Berg (Voorhout, The Netherlands) for kindly providing porcine gut.

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