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Research paper

Thiolated carboxymethylcellulose: in vitro evaluation of its permeation enhancing effect on peptide drugs

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Abstract

The purpose of this study was to evaluate the effect of sodium carboxymethylcellulose (NaCMC) and carboxymethylcellulose–cysteine (CMC-Cys) conjugates on the intestinal permeation of sodium fluorescein (NaFlu) and model peptide drugs, bacitracin and insulin. Cysteine was covalently linked to carbodiimide activated NaCMC. Iodometric titration of the polymer conjugates was used to determine the extent of immobilised cysteine. Permeation studies were performed on guinea pig small intestinal mucosa mounted in Ussing-type chamber. Unmodified NaCMC (1% m/v) significantly improved the transport ratio ($R = P_{app}$ polymer/ P_{app} control) of NaFlu to 1.3 and 1% (m/v) NaCMC conjugated with cysteine further enhanced the permeation. Cysteine conjugation at 3.6, 5.3 and 7.3% (m/m) resulted in *R*-values of 1.4, 1.7 and 1.8, respectively. Decreasing the concentration of CMC–Cys, exhibiting 7.3% (m/m) of immobilised cysteine (CMC–Cys7.3) from 1% (m/v) to 0.5% (m/v) decreased the *R*-value of NaFlu from 1.8 to 1.2. NaCMC at 1% (m/v) in the presence of free cysteine had no significant effect on the *R*-value of NaFlu compared to NaCMC alone. Formulation of fluorescence labelled bacitracin and insulin in unconjugated NaCMC (1% m/v) did not significantly improve the permeation, however in the presence of 1% (m/v) CMC–Cys7.3 a significantly improved permeation was observed ($R = 1.3$). Conjugation at NaCMC with cysteine moieties significantly improves the intestinal permeation of the hydrophilic molecule NaFlu and the model peptide drugs bacitracin and insulin in vitro, therefore this conjugated system maybe useful for peroral administration of peptide drugs in the future.

Keywords: Permeation enhancement; Thiolated carboxymethylcellulose; Tight junctions; Peptide drugs; Ussing-type chamber

1. Introduction

The rapid development in biotechnology and recombinant DNA technology within the last two decades has enabled the communical production of many peptide and protein drugs such as insulin and calcitonin. In order to avoid their parenteral administration which is often difficult, painful and occasionally dangerous, the development of oral dosage forms for these therapeutic agents is a desirable goal for the pharmaceutical industry. Apart from some di- and tripeptides that are absorbed by the intestinal peptide transport systems, the oral bioavailability of peptide and protein drugs, however, is very low. This poor bioavailability is mainly caused by three barriers: the barrier function of the mucus layer covering gastrointestinal epithelia (I) [1], the enzymatic barrier caused by luminally secreted and membrane bound proteases (II) [2,3] and the absorption barrier (III) based on the absorption membrane itself [4]. Attempts to reduce the absorption barrier are based mainly on the co-administration of permeation enhancers such as sodium salicylate and medium-chain glycerides [5,6]. These permeation enhancers are of low molecular size and can therefore be absorbed across the gut causing systemic toxicity [6]. To overcome this problem, mucoadhesive polymers of high molecular mass like sodium carboxymethylcellulose (NaCMC) that are known not to be absorbed from the intestine [7] should therefore be tested for permeation enhancing activity. Furthermore, due to an intimate contact of these mucoadhesive polymers to the absorption membrane, the presystemic metabolism of the peptide or protein within the polymer matrix during absorption will also be reduced [8].

Traditionally, the attachment of mucoadhesive polymers to the mucus layer has been achieved by weak non-covalent bonds. More recently, to improve mucoadhesion some traditional mucoadhesive polymers have been conjugated with cysteine [9,10]. It is generally accepted that these cysteine moieties are able to form stronger covalent bonds with cysteine-rich subdomains of mucus glycoproteins [9]. A

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representative candidate among such thiolated polymers is carboxymethylcellulose–cycteine CMC–Cys, which has been shown to have strongly improved mucoadhesive properties, as well as cohesive properties [9]. The increased cohesive forces of the matrix tablet is believed to be due to the formation of disulfide bonds between the conjugated cysteine moieties of the polymer. Due to this stabilising process within the matrix tablet a controlled drug release could also be provided [9]. Polymeric mucoadhesives have also been shown to inhibit intestinal mucosal proteases, resulting in greater absorption of peptide drugs [3]. In order to eliminate this possible mechanism of proteolytic degradation peptides bacitracin and insulin shown to be stable towards an enzymatic degradation caused by membrane bound peptidases [11,12] were chosen as model drugs.

In this study, we show that the multifunctional polymer NaCMC increases the in vitro permeation of NaFlu across the intestinal mucosa of guinea pig. Furthermore, the permeation of larger analytes, the peptide drugs bacitracin and insulin was only enhanced when the polymer was conjugated with cysteine moieties. Enhanced permeation of these model drugs was correlated with significant decrease in tranepithelial electrical resistance (TEER), suggesting the mechanism of enhancement involves the opening of the tight junctions.

2. Materials and methods

2.1. Synthesis of the CMC–Cys conjugate

The CMC–Cys conjugate was synthesised according to the method previously described by our research group [9,10]. In brief, the covalent attachment of cysteine to sodium carboxymethylcellulose (NaCMC; mol. wt. 1000 kDa Kwizda, Vienna, Austria) was achieved by the formation of amide bonds between the primary amino group of cysteine and the carboxylic acid group of the polymer. NaCMC (10 g) was hydrated in 1 l of demineralised water. To activate the carboxylic acid moieties of the polymer for conjugation, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDAC; Sigma, St. Louis, MO) was added to the hydrated polymer to give a final concentration of 50 mM. The pH of the reaction mixture was adjusted to 6 by the addition of 1 M HCl and L-cysteine (Sigma, St. Louis, MO) was added to give a weight-ratio of 2:1 (polymer:cysteine). The pH of the reaction mixture was maintained at 6 over the 3 h incubation period, under constant mixing at room temperature. The resulting polymer-cysteine conjugate was dialysed in the dark at 10°C, to avoid oxidation of the cysteine moieties. Polymers were dialysed once against 1 mM HCl, twice against the same medium but also containing 1% NaCl and then exhaustively against 0.5 mM HCl. Control polymer was prepared and isolated in the same way as the polymer-cysteine conjugate but EDAC was omitted during the coupling reaction. Samples were lyophilised by drying frozen aqueous polymer solutions at −30°C at 0.01 mbar (Christ Beta 1-8 K; Osterode am Harz, Germany). The polymer–cysteine conjugate and control were stored at 4°C until further use.

2.2. Determination of the thiol group content

The degree of modification was determined by quantifying the amount of thiol moieties on the polymer via iodometric titration at pH 3.0 (1 mM iodine; indicator: starch) [9].

2.3. FITC labelling of bacitracin and insulin

Fluorescein isothiocyanate (FITC, Sigma, St. Louis, MO) was bound to the peptide drugs according to the method previously described by our research group [13]. In brief, 2 mg of FITC dissolved in 1 ml of dimethylsulfoxide was slowly added in aliquot volumes of 25 μl to 40 mg of bacitracin or insulin dissolved in 20 ml of 0.1 M Na_2CO_3. After 8 h of incubation at 4°C, the coupling reaction was stopped by the addition of NH_4Cl, at a final concentration of 50 mM. The resulting bacitracin-FITC (bac-FITC) and insulin-FITC (ins-FITC) conjugates were incubated for 2 h at 4°C, isolated by gel filtration (Sephadex G15, Pharmacia Uppsala, Sweden) [13] and lyophilised as described above.

2.4. Determination of the amount of FITC attached to bacitracin and insulin.

The amount of covalently attached FITC on the peptide drug was determined by measuring the absorbance at 495 nm of 1 mg drug dissolved in 1 ml of demineralised water at pH of 7.4, using a UV/Vis spectrophotometer (Lambda 16, Perkin Elmer; Vienna, Austria). A standard curve of FITC was prepared in demineralised water at pH 7.4.

2.5. Permeation studies

Ussing-type chamber with a surface area of 0.64 cm^2 was used to carry out permeation studies with NaFlu, bac-FITC and ins-FITC. The freshly prepared incubation medium containing 250 mM NaCl, 2.6 mM $MgSO_4$, 10 mM KCl, 40 mM glucose and 50 mM $NaHCO_3$ was buffered with 40 mM N-(2-hydroxyethyl)piperazine-N′-(2-ethane-sulfonic acid) (HEPES, Sigma, St. Louis, MO). The pH was adjusted with 5 M NaOH to 7.4. The first 10 cm of the small intestine (duodenum) of the guinea pig was excised immediately after sacrificing the animal and mounted in the Ussing-type chamber, without stripping off the underlying muscle layer. The donor and acceptor compartments of the Ussing-type chamber were filled with 1.0 ml of the incubation medium pH 7.4.

Permeation studies were performed in an atmosphere of 95% O_2 and 5% CO_2 at 37°C and were started 15–20 min. after the mounting of the tissue. The solution in the donor chamber was replaced with incubation medium containing

either 0.001% (m/v) NaFlu, 0.1% (m/v) bac-FITC or 0.1% (m/v) ins-FITC with or without test polymer at various concentrations. Over a 3 h incubation period 200 μl samples were taken from the acceptor chamber every 15 min and the volume was replaced by the same medium equilibrated at 37°C. The permeation enhancing effect of NaCMC and the CMC-cysteine conjugate was evaluated by measuring the amount of permeated test compound in the acceptor chamber using a fluorimeter (SLT; Spectra Fluor; Tecan, Austria). Cumulative corrections were made for the previously removed samples in determining the total amount permeated.

2.6. Viability studies

After permeation studies the medium was removed from the donor chamber and 1 ml trypan blue dye was added and the mucosa was incubated for 30 min. Microscopic investigations demonstrated, that the mucus was still present and that the viability of the intestinal membrane was guaranteed, as no blue colour was detectable within the cells. These observations were in good agreement with the results of viability studies previously published by other research groups [14].

2.7. Measurement of the transepithelial electrical resistance

A Millicell® ERS meter (Millipore Corp., Bedford, MA) connected to a pair of side by side electrodes was used to monitor the effect of the polymers on the TEER of the intestinal mucosa. Measurements were performed every 5 minutes before applying the polymer and then every 15 min within 3 h.

2.8. Enzymatic stability of insulin-FITC by HPLC-analysis

A 100 μl sample of the donor and acceptor medium of the Ussing-type chamber was withdrawn every 60 min over a 6 h period and 20 μl was analysed by HPLC (series 200 LC; Perkin-Elmer, Norwalk, CT). The HPLC-analysis was carried out in a slightly modified way as described previously by our research group [15,16]. Analysis of the samples by reversed phase HPLC were conducted using a HPLC and a diode array detector (Perkin-Elmer 235C). Samples were eluted from a Nucleosil 100–5 C18 column (250 × 4 mm) at 40°C, with a linear gradient from 91% A/9% B to 39% A/61% B (eluent A: 0.1% trifluoroacetic acid in water; eluent B: acetonitrile) at a flow rate of 1.0 ml/min for 22 min. The eluate was monitored at 220 nm.

2.9. Data analyses

Apparent permeability coefficients (P_{app}) for NaFlu, bac-FITC and ins-FITC were calculated according to the following equation

$$P_{app} = Q/A^{*}c^{*}t$$

where P_{app} is the apparent permeability coefficient (cm/s), Q is the total amount permeated throughout the incubation time (μg), A is the diffusion area of the Ussing-type chamber (cm^2), c is the initial concentration of the marker in the donor compartment ($\mu g/cm^3$), and t is the total time of the experiment (s).

Transport enhancement ratios (R) were calculated from P_{app} values by

$$R = P_{app}(\text{sample})/P_{app}(\text{control})$$

Statistical data analyses were performed using the student t-test with $P < 0.05$ as the minimal level of significance.

3. Results

3.1. Synthesis and characterisation of the CMC-Cys conjugate

The CMC-Cys conjugates, as shown in Fig. 1, have already been characterised in previous studies [9,10]. These studies showed that the optimum pH for the coupling reaction of cysteine to NaCMC-polymer was at pH 6 [9,10]. Lower coupling at pH above 6 is believed to be due to the oxidation of the sulfhydryl compound during the coupling reaction [9]. In this study iodometric titration showed that the conjugate efficiency of NaCMC was 7.3% (m/m). This conjugate was subsequently used in these studies. Control polymer was prepared and isolated in the same way as the CMC-Cys conjugate, but EDAC was omitted during the coupling reaction. Iodometric titration of this control showed that 0.0% cysteine was conjugated. Previous studies demonstrated that the polymer-cysteine conjugate remained stable after lyophilisation and in aqueous solutions of pH 5.0 and lower [9].

3.2. Influence of NaCMC and CMC-Cys conjugate on drug permeation

The permeation enhancing effect of NaCMC and CMC-Cys7.3 on NaFlu and peptide drugs, bacitracin and insulin was evaluated in vitro with the small intestinal tissue isolated from guinea pig mounted in a Ussing-type chamber. Permeation studies with NaFlu in the presence of 1%

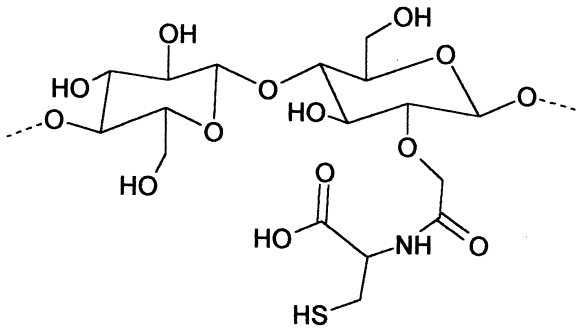

Fig. 1. Presumptive chemical substructure of the carboxymethylcellulose-cysteine conjugate.

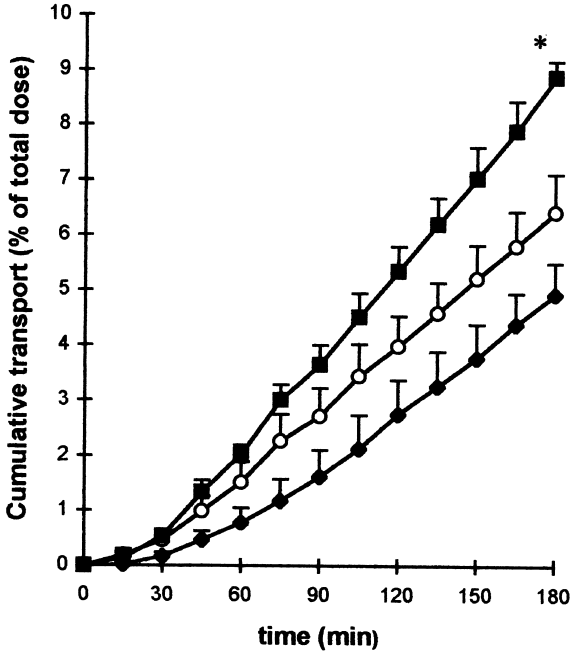

Fig. 2. Transport of NaFlu across small intestinal mucosa of guinea pigs. Transport data are expressed as percentage of the total dose of NaFlu applied to the luminal side of the mucosa. Control without polymer ◆; 1% (m/v) NaCMC ○; 1% (m/v) CMC-Cys7.3 ■; (mean ± SD; $n = 3$); * differs from control without polymer, $P < 0.001$.

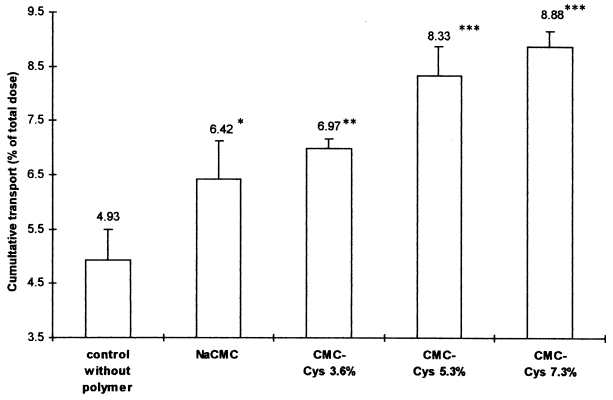

Fig. 3. Comparison of the effect of 1% (m/v) NaCMC derivatives with increasing amounts of covalently attached cysteine on the cumulative transport of NaFlu across the small intestinal mucosa of guinea pigs after a time period of 180 min. Each point represents the mean ± SD of three experiments * differs from control without polymer, $P < 0.1$; ** differs from control without polymer, $P < 0.01$; *** differs from control without polymer, $P < 0.001$.

NaCMC led to a significantly improved permeation of the model drug (Fig. 2). The permeation of NaFlu was further improved by CMC-Cys7.3 (Fig. 2). In order to determine the effect of lowering the amount of cysteine moieties without decreasing the amount of NaCMC in the formulation, CMC-Cys7.3 was mixed with unconjugated NaCMC as described in Table 1. It was demonstrated that decreasing the amount of cysteine moieties in this way resulted in lower permeation of NaFlu (Fig. 3). CMC-Cys7.3 at 1% (m/v) significantly improved the permeation of the model peptide drugs bac-FITC and ins-FITC (Figs. 4 and 5, mean $n = 3$; $P < 0.05$). The according permeation enhancing ratios are depicted in Table 2.

Furthermore, the concentration of the CMC-Cys7.3 polymer applied to the donor chamber of the Ussing-type chamber was also shown to influence the permeation of NaFlu. Lowering the CMC-Cys7.3 concentration from 1 to 0.5% resulted in the permeation of NaFlu to decrease from 1.8 to 1.2 (Fig. 6). The permeation enhancing ratio for NaFlu in the presence of 0.5% CMC-Cys7.3 was the same as for 1% of unmodified NaCMC, although the concentration was the half of the unmodified polymer. The permeation enhancing effect of a mixture of CMC and the corresponding amount of free unconjugated cysteine was in the range of unmodified NaCMC (data not shown). Additionally, the permeation enhancing effect of a CMC-Cys conjugate, which had been hydrated and completely oxidised at pH 7.4, was also in the range of the unmodified polymer (data not shown). Hence the presence of immobilised thiol moieties on the polymer is a crucial factor for the underlying mechanism of improved permeation.

3.3. Influence of the molecular radius of peptide drugs on permeation

To illustrate the influence of the molecular radius (m.r.) on the permeation via the paracellular pathway NaFlu (m.r. 5.5 Å) – a widely used marker for the paracellular way of absorption – bacitracin-FITC (m.r. 7–10 Å) and insulin-FITC (m.r. 35 Å in minimum) were used as model drugs. On average, approximately 0.04 mol and 0.33 mol FITC were bound to one mole bacitracin and insulin, respectively.

Table 1
Preparation of test-polymers with increasing amounts of covalently attached cysteine; CMC-Cys7.3 = carboxymethylcellulose-cysteine conjugate exhibiting an amount of 7.3% (m/m) of immobilised cysteine

Test-polymer	CMC-Cys7.3(mg)	NaCMC(mg)	Percentage of covalently attached cysteine in test-polymer(%)	μmol Thiol groups per g test-polymer
CMC-Cys7.3	10.0	–	7.3 ± 0.3	602.29 ± 24.7
CMC-Cys5.3	7.3	2.7	5.3 ± 0.1	437.28 ± 8.23
CMC-Cys3.6	5.0	5.0	3.6 ± 0.1	298.36 ± 8.62
Control	–	10.0	0.0 ± 0.0	0.00 ± 0.0

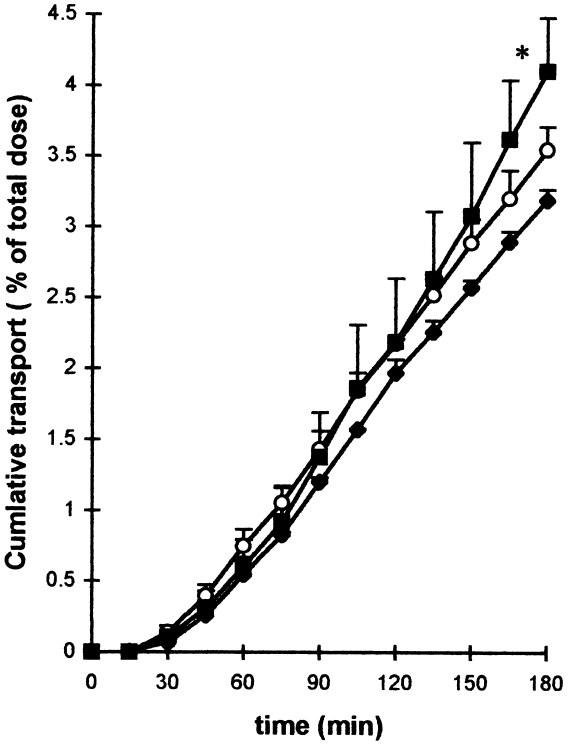

Fig. 4. Transport data of bac-FITC across small intestinal mucosa of guinea pigs are expressed as percentage of the total dose of bac-FITC applied to the luminal side of the mucosa. Control without polymer ◆; 1% (m/v) NaCMC ○; 1% (m/v) CMC–Cys7.3 ■; (mean ± SD; $n = 3$). * differs from control without polymer, $P < 0.001$.

Permeation studies showed that the permeation rate was greatest for NaFlu (7.16×10^{-6}cm/s) and considerably lower for the higher m.r. molecules of bac-FITC (4.51×10^{-6}cm/s) and ins-FITC (4.23×10^{-6}cm/s) (Figs. 2, 4, 5).

This data demonstrates that peptide drugs with a high molecular radius pass through the tight junctions to a lower amount than substances with smaller molecular radii. The apparent permeation ratios are shown in Table 2.

3.4. Metabolic stability of ins-FITC

To determine if there is any proteolytic activity towards ins-FITC during its absorption across the guinea pig mucosa, samples from the acceptor and donor sides were analysed by HPLC. No insulin degradation products appeared after 180 min. Furthermore, after 360 min only a negligible degradation of insulin-FITC was detected. The results confirmed that insulin-FITC is not degraded by membrane bound peptidases during the permeation process.

3.5. Measurement of the TEER

TEER measurements were performed throughout all permeation studies and served as an indicator for the opening of the tight junctions. Fig. 7 demonstrates a significant decrease in TEER after the addition of NaCMC or CMC-Cys7.3 to the donor chamber. Incubating the small intestinal mucosa in the Ussing-type chamber for a time period of 180 min in the presence of either 1% NaCMC or 1% CMC–Cys7.3, caused the TEER to decrease significantly by 10.6 ± 1.4 and 18.1 ± 0.2% (mean ± SD; $n = 3$), respectively, compared to control without polymer. Unconjugated NaCMC (1% m/v) in the presence of free cysteine (7.3% m/m) and 1% (m/v) CMC–Cys7.3 conjugate that was completely oxidised to eliminate free thiol moieties were assayed for their ability to reduce TEER in vitro. Both preparations were shown to reduce the TEER, however not significantly different from the unmodified NaCMC. These results are in good agreement with the permeation studies where an improved permeation was accompanied with a decrease in TEER. In addition, a recovery of TEER was observed by removing the NaCMC and CMC-Cys conjugate after 180 min incubation

4. Discussion

This study demonstrates that the mucoadhesive polymer NaCMC displays a permeation enhancing effect. With the addition of the anionogenic polymer NaCMC a 1.3-fold improved permeation accompanied by a decrease of the TEER-values could be monitored for all tested model drugs. As the high binding capability of anionogenic polymers for multivalent actions is generally accepted, the Ca^{2+}

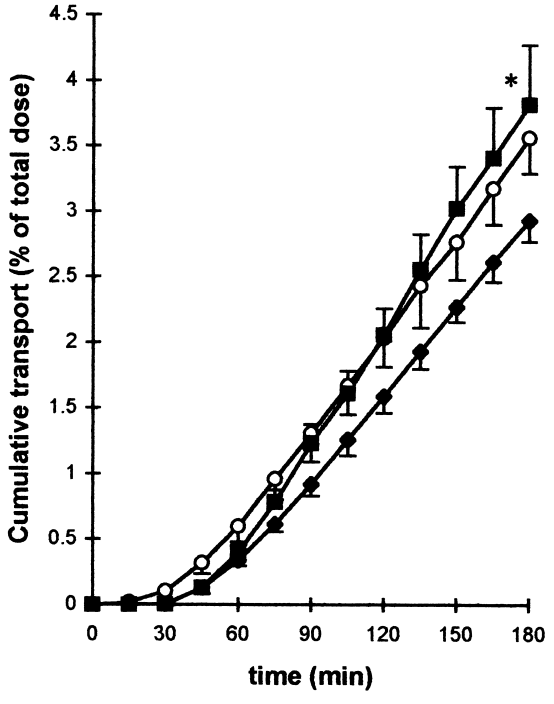

Fig. 5. Transport of ins-FITC across small intestinal mucosa of guinea pigs. Transport data are expressed as percentage of the total dose of ins-FITC applied to the luminal side of the mucosa. Control without polymer ◆; 1% (m/v) NaCMC ○; 1% (m/v) CMC–Cys7.3 ■; (mean ± SD; $n = 3$). * Differs from control without polymer, $P < 0.05$.

Table 2
Comparison of the influence of NaCMC and CMC–Cys on the apparent permeability coefficient (P_{app}) for NaFlu, bac-FITC and ins-FITC across the intestinal mucosa of guinea pigs

Test compound	Polymer	Apparent permeability coefficient ($P_{app} \times 10^{-6}$ (cm/s)), means ± SD, $n = 3$	Enhancement ratio (P_{app} polymer/P_{app} control)
NaFlu	Without polymer	7.16 ± 0.83	1
	1% (m/v) NaCMC	9.35 ± 1.01	1.30
	1% (m/v) CMC-Cys3.6	10.14 ± 0.29	1.41
	1% (m/v) CMC-Cys5.3	12.12 ± 0.80	1.69
	1% (m/v) CMC-Cys7.3	12.92 ± 0.41	1.80
	0.5% (m/v) CMC-Cys7.3	8.52 ± 0.64	1.18
bac-FITC	Without polymer	4.51 ± 0.22	1
	1% (m/v) NaCMC	5.33 ± 0.32	1.18
	1% (m/v) CMC-Cys7.3	5.98 ± 0.54	1.32
ins-FITC	Without polymer	4.23 ± 0.23	1
	1% (m/v) NaCMC	5.20 ± 0.40	1.22
	1% (m/v) CMC-Cys7.3	5.55 ± 0.65	1.31

depletion by NaCMC could be assumed to open the tight junctions. This mechanism was shown in previous studies for PCP [17] and could possibly be the underlying mechanism for the improved permeation enhancing properties of the anionogenic polymer NaCMC.

Moreover, the covalent attachment of cysteine to NaCMC led to a further improvement of the permeation of NaFlu, bacitracin-FITC as well as insulin-FITC. This enhanced permeation was accompanied by a decrease of the TEER-values indicating the loosening of the tightness of intercellular junctions, i.e. the opening of the paracellular route across the epithelium for otherwise non-absorbable hydrophilic compounds such as peptides. The molecular and cellular mechanism concerning the gate fence function of the tight junctions is still not completely understood. Opening and closing of the tight junctions seem to be mediated also by various proteins, e.g. occludin, ZO1 and ZO2 [18]. Occludin located in the epithelial cells expressing an extracellular (I), membrane (II) and cytoplasmic region (III) is somehow involved in the regulating mechanism of the tight junctions [19].

The alteration of occludin and subsequently the opening of the intercellular junctions, can be explained by the following processes. On the one hand, phosphorylation of tyrosine groups from the extracellular region by protein kinases causes a decrease of the TEER-values which indicates an increase of the permeability of the tight junctions

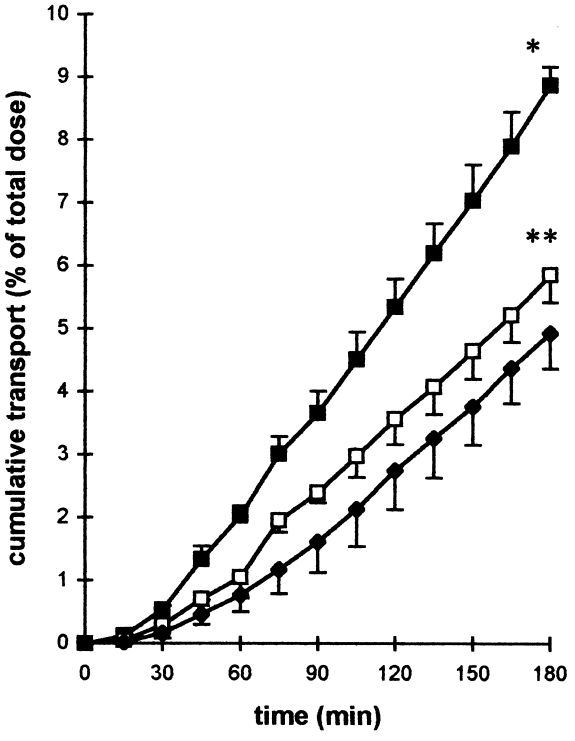

Fig. 6. Influence of different concentrations of the CMC–Cys7.3 conjugate on the permeation of NaFlu across small intestinal mucosa. 1% (m/v) CMC–Cys7.3 ■; 0.5% (m/v) CMC–Cys7.3 □; 0% (m/v) CMC–Cys7.3 ◆; (means ± SD; $n = 3$); *differs from control without polymer, $P < 0.001$; **differs from control without polymer, $P < 0.01$.

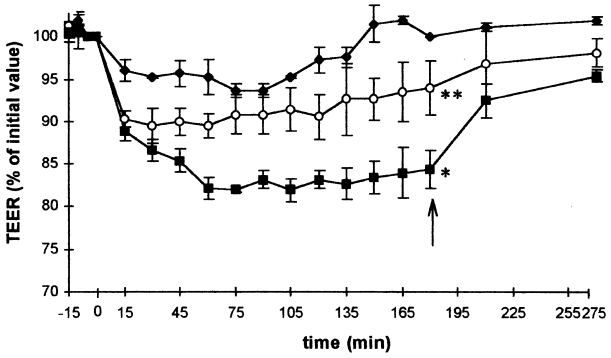

Fig. 7. Effect of 1% (m/v) NaCMC and CMC–Cys7.3 on the TEER of small intestinal mucosa of guinea pigs at pH 7.4. Each point represents the mean ± SD of at least three experiments. Keys: control without polymer ◆; 1% (m/v) NaCMC ○; 1% (m/v) CMC–Cys7.3 ■; reversibility experiment started as indicated after 180 min of incubation; (means ± SD; $n = 3$); * differs from control without polymer, $P < 0.001$; **differs from control without polymer, $P < 0.05$.

[20,21]. On the other hand, a dephosphorylation mediated by protein tyrosine phosphatase (PTP) leads to a closing process followed by an increase of the TEER-values [22]. The inhibition of PTP by specific inhibitors such as vanadate or pervanadate causes an enhanced opening of the tight junctions. PTP1B, a representative member of the PTP-family comprising more than 30 members, was chosen as model enzyme for studies concerning the activity of PTP [23]. The results demonstrated that the active site cysteine residue of PTP, Cys 215, is essential for the activity of this phosphatase [24]. Studies with glutathione showed that Cys 215 was able to form a mixed disulfide causing an inactivation of PTP1B [25]. This inactivation stopped the dephosphorylation of tyrosine-residues and led thereby to an increase in tight junction permeability [20]. Being aware of the important role of the Cys 215 for the activity of the PTP, an inactivation could be reached by the CMC-Cys conjugate via the formation of a mixed disulfide bond with the Cys 215. This inactivation would consequently result in a loosening of the tightness of the tight junctions and thereby to an improved permeability for peptide drugs. This presumptive thiol dependent PTP-inactivation by CMC-Cys might explain the improved permeation enhancing effect of the CMC-Cys conjugate. Furthermore this permeation enhancing effect could also be monitored for an anionic thiomer namely polycarbophil-Cys as well as for the cationic thiomer chitosan-Cys conjugate [13,26]. This substantiates the theory concerning the essentiality of the covalent attachment of thiol groups onto a polymer. Hence, an interaction between the thiol group of the conjugate and the Cys 215 of the PTP is able to take place. In order to verify this theory detailed studies should be subject of further investigations.

Moreover, the mucus displays a hindrance which permeation enhancers must overcome in order to reach the epithelial cell surface. In particular for NaCMC and CMC-Cys this effect could lead to a strong reduction in their permeation enhancing properties. Studies on Caco 2 monolayers with and without a covering mucus layer, for instance, demonstrated a dramatic decrease of the permeation enhancing effect of chitosan in the presence of the mucus [27]. This experiment pointed out that the barrier function of the mucus is a major limiting factor for the permeation enhancing effect of polymers. All the studies described here, however, were carried out on the small intestine of guinea pigs covered with a mucus layer being analysed with a microscope, which is a crucial difference to the widely used Caco 2 monolayer model. Although the limiting barrier function of the mucus was present the results showed that the permeation enhancing effect of NaCMC can even be significantly improved due to the covalent attachment of cysteine to the polymer.

This circumstance can, on the one hand, be supported by an improved mucoadhesion based on a thiol/disulfide exchange reaction between the thiomer and the thiol groups of the glycoproteins of the mucus [9]. On the other hand, based on an oxidation process, thiol groups within the matrix tablets are able to form disulfide bonds subsequently leading to an improved structural stability of the drug delivery system [10]. Hence, the thiolated polymer can directly act in high concentrations of about 15% (m/v) at the area where the drug is released and absorbed [10]. Additionally, the permeation enhancing effect of the polymer will also take place within this area. Due to the direct correlation between the concentration and the permeation enhancing effect a much higher effect can be expected. In contrast permeation enhancers with a low molecular weight e.g. sodium glycocholate, sodium deoxylate, sodium taurocholate and EDTA showing a permeation ratio for insulin of 2.5, 4.18, 1.03 and 2.00, respectively, will not remain concentrated at the area of absorption [28]. Due to their small molecular weight they will also be absorbed which could lead to some extent to systemic side effects. Taking all these aspects into consideration the CMC-Cys7.3 conjugate with a permeation ratio of 1.31 for insulin seems to be a promising alternative to the well established permeation enhancer.

5. Conclusion

Within this study, the mucoadhesive polymer NaCMC could be revealed as a promising permeation enhancer for the paracellular route of absorption which is favoured by hydrophilic compounds of high molecular mass such as peptide drugs. This permeation enhancing effect could even be strongly improved due to the covalent attachment of cysteine to the polymer. The capability of (thiolated) NaCMC, on the one hand, and its mucoadhesive properties providing an intimate contact of the delivery system with the absorption membrane, on the other hand, should render these polymers useful especially for the noninvasive administration of peptide drugs.

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