Surface hydrophilic modification of chitosan copolymer based self-assembly polyelectrolyte nanocomplex for enhanced drug absorption

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Introduction. So far, numerous nanocarriers have been extensively used to protect the proteins from enzymatic degradation, with mucoadhesive nanocarriers designed to prolong the retention in the gastrointestinal tract in order to enhance the in vivo absorption. It has yet to be carefully noticed that the adhesion of drug loaded nanoparticles to the mucus cannot offer a valid option for drugs reaching their target across the mucus layer due to mucus layer clearance or leaking from the carrier. For a more efficient transport of drugs to permeate the mucus gel layer subsequently reaching the epithelium, mucopenetrating formulations on the other hand are utilized [1]. Nevertheless, the subsequently transport across the intestinal epithelium still remains a barrier because of the low affinity of slippery NPs with a lipophilic and negatively charged cell membrane. Therefore, it is desirable to design NPs which can cross the two barriers meanwhile. However, it was noticed that mucus layer penetration and epithelium layer permeation require contradictory surface properties, that is, a hydrophilic and neutrally charged surface benefit for the mucus barrier, whereas a hydrophobic and cationic surface preferentially for the epithelial barrier[2]. Thus, it is still not clear, surface hydrophilicity or hydrophobicity, which contributed most to the oral absorption of insulin and whether the nanoparticles with both properties can provide better gains for the absorption.

Purpose. Using CS as the nanocarrier, the objective of this study is to elucidate the contribution of each strategy, mucus penetration and mucoadhesion, on the oral absorption of insulin and the essential for these two strategies combination.

Methods. First of all, CS-g-mPEG with different mPEG graft ratios was synthesized, the optimized CS-g-mPEG copolymer graft ratio was confirmed via a series of in vitro and in vivo studies of CS-g-mPEG based insulin nanocomplex including particle size, surface hydrophobicity, mucus interaction, ex vivo permeation and in vivo efficacy studies. Thereafter, with newly synthesized mPEG-CS-glyceryl monocaprylate (GMC) copolymers, the added hydrophobic modification on the in

vitro and in vivo properties of the mPEG-CS based PECs was explored.

Results

CS-g-mPEG and mPEG-CS-GMC The copolymers were synthesized by grafting activated mPEG to the backbone of chitosan via a classical amide formation with EDC. The successful synthesis of the copolymer was confirmed using ^{1}H-NMR and FT-IR. Considering the physiological structure of intestinal tract, hydrophilic modified nanocarriers will facilitate mucus penetration, thereafter hydrophobic property might be helpful in enhancing epithelium cell uptake. Thus, it is absolutely essential to figure out whether hydrophobic modification can make further contribution in absorption on the basis of the optimized mPEG graft ratio on CS. Our previous study has confirmed that hydrophobic modification of CS with GMC at graft ratio 10%（CS-$GMC_{10\%}$ copolymer）presented the best epithelial cells uptake and in vivo absorption[3-4]. Therefore, by keeping GMC graft ratio at 10%, mPEG-CS-$GMC_{10\%}$ copolymer was synthesized.

Influence of mPEG graft ratio on the physiochemical properties of the PEC It was noted that no significant difference in particle size, zeta potential and EE was found when mPEG graft ratio was increased from 5% to 10% (p>0.05). And, further increasing mPEG graft ratio to 18% caused no statistical decrease in particle size, zeta potential compared with CS-$mPEG_{10\%}$ group (p > 0.05), but a significant decrease in EE was observed (p<0.05).

Influence of mPEG graft ratio on the stability of the PEC The non-modified CS/Insulin PEC and three mPEG graft ratio based PECs all exhibited excellent particle stability in SIF containing trypsin, with no significant variation of size (p>0.05). A different situation was observed in the gastric medium containing enzymes where the presence of mPEG attached to CS backbone was found to improve the stability of the PECs compared with non-modified CS/Insulin PECs(p<0.05). In agreement with the results from stability studies, the protective effect of PECs on insulin from trypsin degradation was mPEG graft ratio dependent. When the mPEG graft ratio was 5%, the amount of remaining insulin had no significant difference with that of CS/insulin PECs group. As the mPEG graft ratio increased to 10%, a significant improvement in insulin remaining amount was observed. Though the mPEG graft ratio reached up to 18%, the insulin remaining ratio was only 30% after 3h, indicating the protective effect provided by PECs was limited.

Influence of mPEG graft ratio on the hydrophilicity of the PEC. Compared to CS based PEC, the Rose Bengal binding constants of all the CS-mPEG based nanocomplexes decreased significantly

(p<0.05), indicating a remarkable increase in hydrophilicity of the PECs. As to the influence of mPEG graft ratio, no significant difference in surface hydrophilicity was found between the CS-mPEG$_{5\%}$ and the CS-mPEG$_{10\%}$ groups (p > 0.05). In contrast, a significant increase in surface hydrophilicity was achieved when further increasing mPEG graft ratio to 18% (p < 0.05). Consequently, it can be concluded that mPEGylation of chitosan increased surface hydrophilicity of the PECs in an mPEG graft ratio dependent manner.

Influence of mPEG graft ratio on the interaction with mucin. In agreement with its mucoadhesive properties, CS based PECs exhibited a marked increase in mucoadhesion, and the influence of mPEGylation on the mucoadhesion of the PEC was mPEG graft ratio dependent. Compared with CS based PECs, the interaction of CS-mPEG$_{5\%}$ PEC with mucin decreased remarkably (p<0.05). And further decrease in mucoadhesion was observed when mPEG graft ratio increased from 5% to 10% (p<0.05), although they presented similar hydrophilicity based on Rose Bengal assay. However, when the grafting ratio of mPEG increased from 10% to 18%, despite of the significant increase in surface hydrophilicity, large aggregates were formed with mucin, which was even similar to that formed with CS PECs ($p>0.05$), implying the enhanced interaction with mucin at this mPEG graft ratio.

Influence of mPEG graft ratio on the drug release behavior. The release profile of insulin previously from CS-mPEG$_{5\%}$/Insulin PEC was similar to that of control CS/Insulin PEC (f_2 >50). On the other hand, the significantly slower release profile of insulin encapsulated in CS-mPEG$_{10\%}$/Insulin PEC and CS-mPEG$_{18\%}$/Insulin PEC (f_2 <50) compared with non-modified CS PEC indicated that the extra mPEG coating indeed facilitated the retention of the insulin in the nanocomplexes. It should also be pointed out that an increase in the mPEGylation degree of chitosan (18%) led to a significant decrease in the percentage of insulin released in the initial phase (10%). In agreement with the observed effect of the mPEG graft ratio of CS on the encapsulation efficiency of insulin, the extra mPEG coating also affects the release behavior of the resulting nanocapsules, which could be related to the different organization of the coating achieved as amounts of mPEG chains bound on CS surface increased.

Influence of mPEG graft ratio on the in vivo hypoglycemic effect. Compared with oral CS/Insulin nanocomplexes, all the CS-mPEG/Insulin nanocomplexes investigated exhibited significantly enhanced hypoglycemic effect in 4 h (p<0.05). Among them, a notable drop in blood glucose was observed after oral administration of CS-mPEG$_{10\%}$/Insulin PEC during 1 to 6 h. Compared with CS based PEC, CS-PEG based PEC led to further enhanced absorption and the extent was mPEG graft ratio dependent.

Whereas, CS-mPEG$_{10\%}$/Insulin PEC, which presented the lowest interaction with mucin, exhibited the highest relative pharmacological availability of 5.2%, 3.5-fold higher than non-modified CS/Insulin PEC, 1.4-fold higher than CS-mPEG$_{5\%}$/Insulin PEC. However, no further increase in absorption was observed with CS-mPEG$_{18\%}$/Insulin PEC despite of its significantly higher surface hydrophilicity.

Influence of hydrophobic modification on the properties of mPEG-g-CS based PEC. mPEG$_{10\%}$-CS-GMC$_{10\%}$ based PEC exhibited no significant change in the particle size for up to 2 h of incubation (p > 0.05). Surface hydrophilicity was decreased by GMC modification, but was still much higher than that of CS-GMC$_{10\%}$/Insulin PEC. CS-GMC$_{10\%}$ based PEC presented stronger mucoadhesive property, while the mucoadhesion decreased significantly via PEG modification (mPEG$_{10\%}$-CS-GMC$_{10\%}$ based PEC). mPEG$_{10\%}$-CS-GMC$_{10\%}$ based PEC exhibited similar release behavior to that of CS-mPEG$_{10\%}$ PEC (f_2 >50) with insulin release about 68-70% for 6 h in SIF. To further figure out the influence of surface hydrophilicity/mucin interaction on the permeability of loaded insulin through different intestine segments including duodenum, jejunum and ileum, the permeation amount and permeation coefficient of insulin in nanocarriers with different surface properties, CS-Insulin PEC, CS-mPEG10%/Insulin PEC, CS-GMC10%/Insulin PEC, mPEG10%-CS-GMC10%/Insulin PEC, were investigated as described previously [5]. In both duodenum and jejunum, during the investigated 2h, CS-mPEG10%/Insulin PEC and mPEG10%-CS-GMC10%/Insulin PEC exhibited comparable permeability, with a 3.5-3.7 and 1.6-1.8 fold enhanced Papp value than that of free insulin solution and CS PEC, respectively ($p<0.05$), while lower permeation amount was observed for CS-GMC10%/Insulin PEC($p<0.05$), implying surface property of the nanocarriers influences insulin permeability through duodenum and jejunum, with hydrophilic nanocarriers preferred. In contrast, in ileum, probably due to its higher thickness, no statistical difference in permeation amount among CS-mPEG10%/Insulin PEC, CS-GMC10%/Insulin PEC, mPEG10%-CS-GMC10%/Insulin PEC groups were found ($p>0.05$).

Conclusion. The in vitro and in vivo properties of CS-g-mPEG based insulin nanocomplex was PEG graft ratio dependent and the best absorption was achieved at PEG graft ratio 10%. CS-mPEG$_{10\%}$/Insulin PEC group presented better permeation in duodenum and jejunum than that of CS-GMC$_{10\%}$/Insulin PEC group. In agreement with the in vitro permeation data, CS-mPEG$_{10\%}$/Insulin PEC group presented better therapeutic effect than that of CS-GMC$_{10\%}$/Insulin PEC group. Further modification of CS-mPEG$_{10\%}$ with GMC led to prolonged therapeutic effect but no statistical difference

in pharmacological availability.

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References:

[1] Pereira de Sousa I, Steiner C, Schmutzler M. Mucus permeating carriers: formulation and characterization of highly densely charged nanoparticles. Eur J Pharm Biopharm, 2015;97(Pt A): 273-279.

[2] Koziolek M, Grimm M, Schneider F. Navigating the human gastrointestinal tract for oral drug delivery: Uncharted waters and new frontiers. Adv Drug Deliv Rev. 2016; 101: 75-88.

[3] Wang L, Li L, Sun Y. In vitro and in vivo evaluation of chitosan graft glyceryl monooleate as peroral delivery carrier of enoxaparin. Int J Pharm, 2014; 471(1-2): 391-399.

[4] Wang L., Sun Y, Shi C (2014). Uptake, transport and peroral absorption of fatty glyceride grafted chitosan copolymer-enoxaparin nanocomplexes: Influence of glyceride chain length. Acta Biomaterialia.2014; 10(8): 3675-3685.

[5] Zhang X, Cheng H, Dong W. Design and intestinal mucus penetration mechanism of core-shell nanocomplex. J Control Release.2018; 272: 29-38.

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