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Oral delivery of proteins and peptides by mucoadhesive nanoparticles

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ABSTRACT

It seems that an ever growing tendency is necessary for the development of a strategy that can not only protect the peptides and proteins from degradation by the enzymes but also can improve their absorption without affecting biological activity. Delivery of peptides and proteins into the oral cavity remains an interesting approach, however for its availability some distinctive problems should be addressed. A wide variety of carriers have been investigated for delivery of peptides and proteins for the protection of their formulation and structure against the enzymatic environment of the gastrointestinal tract.

Keywords: *Nanotechnology, Mucoadhesive nanoparticles, Oral delivery.*

1. INTRODUCTION

During recent years, nanoparticles have been reported and used as potential systems for delivery of therapeutic peptides and proteins to specific target through a different route of administrations. Some groups of nanoparticles have been reported to increase the peptides and proteins' oral bioavailability. Nanoparticles can improve the stability of peptides and proteins, control their release, as well as decrease the toxicity in the peripheral normal tissues. The peptide or protein can be dissolved, entrapped, encapsulated, adsorbed, or attached into nanoparticles for the efficient delivery into the target site. Besides, the surface of nanoparticles can be modified for opsonization [1-4]. Polymeric nanoparticles are defined as solid particles with different size ranging from 10 to 1000 nm, allowing the encapsulation of the drugs, and protecting them against hydrolytic and enzymatic degradation [5-8]. It was demonstrated that oral administration of insulin-loaded nanoparticles can reduce blood glucose level in a diabetic animal model up to 14 days, stimulating research into nanoparticulate systems for oral delivery of peptides, potentially [9]. Over the past few years, not only improvement of oral peptide bioavailability, but also the development of mucosal vaccines have become more prominent. Nasal and oral dosage forms increase patient convenience and facilitate frequent boosting, necessary to obtain the best immune response. Epithelial surfaces are the primary port of entry for many bacterial or viral pathogens, and mucosal immunity can be considered as a major protective barrier. One limitation for oral delivery by nanoparticles is the requirement that particles need to be absorbed from the gastrointestinal tract (GI) with a sufficient extent and rate [10].

Physiological factors that affect the absorption of nanoparticles in the body and their interdependence with physicochemical characteristics of the polymeric vehicle are not well understood and will discuss in more detail below. Since, polymers in colloidal drug delivery systems are absorbed into the organism, thereby additional requirements such as biocompatibility and biodegradability need to be considered. Furthermore, size and encapsulation efficiency depend on the polymers used in the formulation of the nanoparticles and thus the use of novel biomaterials combined with all these considerations might be of general interest for the development of nanoparticulate carriers for efficient mucosal peptide delivery [11]. Kafka and co-workers fabricated poly (ethylcyanoacrylate)-based polymeric nanoparticles and loaded with sterilant D-Lys6-Gonadotropin releasing hormone (D-Lys6-GnRH). The simulated gastric juice, artificial intestinal fluid and brushtail possum plasma were used as *in vitro* conditions. In all the three conditions, the D-Lys6-GnRH was intact, while the percent of bioactive component realeasing was different, < 5% of the it released after 6 h in simulated gastric juice and artificial intestinal fluids versus 60% which is released in brushtail possum plasma over 1 h. *In vivo* administration of nanoparticles into the brushtail possums' caecum exhibited that an adequate amount of the bioactive component is released into the systemic circulation. Moreover, the luteinized-hormone releasing supported that an extensive amount of it was available in the pituitary gland [12].

2. MUCOADHESIVE NANOPARTICLES

Significant work is being done for the modification of nanoparticles for a better understanding of all the factors influencing mucoadhesion capacity along with their subsequent absorption by the GI tract. Florence et al. have demonstrated the role of different biodegradable polymers in improving the bioavailability of nanoparticles. In another study accomplished by this group, about 10% of invasin-coated latex nanoparticles (at a size of about 500 nm) reached into the systemic circulation through the oral administration in rats [13]. They demonstrated the presence of an optimum emulsion size for an effective nanoparticles entrapment within the mucosal layer and for epithelial cells trapping particles to operate endocytic processes [14]. Nanoparticles prepared from lipophilic polystyrene, PLA-PEG and mucoadhesive chitosan were observed within both epithelial and Peyer's patches following interduodenal administration of drug agents [15]. Peyer's patches are the follicles of lymphoid tissue containing M-cells, having an important function in the uptake of particles. The size and surface charge are the most important factors affecting the uptake of particles by M-cells [16].

Polymeric nanoparticles have been established extensively to elevate the biological half-life of therapeutic proteins and peptides by entrapment and encapsulation. In addition, by encapsulation of drugs these particles facilitate the target delivery and avoid the interaction between drug and healthy cells. In this paper recent examples of the different applied pharmaceutical-based techniques for effective delivery of therapeutic proteins and peptides will be discussed. A comprehensive search of the literature was performed using a selection of various electronic search databases such as PubMed, Web of Science, google scholar, and Science Direct. This study enlists a number of search terms and advanced search by combining all fields (including title, abstract, keywords and titles). Numerous studies listed in this paper support the beneficial effects of polymeric-based techniques for delivery of therapeutic proteins as promising non-toxic drug carriers. It can be smoothly functionalized for off opsonisation, and therefore has shown reduced toxicity towards the non-target areas (peripheral tissues) [17].

Previous *in vitro/in vivo* studies indicated that gonadotropin releasing hormone containing Poly (ethylcyanoacrylate) (PECA) nanoparticles can ease the uptake of sufficient therapeutic levels of GnRH from the caecum and release it into systemic circulation [6, 12].

The mucoadhesive nanoparticles play a key role as an innovative drug delivery system which increases the residence time of the drug at the target site and strongly adhere to the mucous membrane of gastrointestinal tract. This advantage of mucoadhesive nanocarriers could reduce clearance and improve bioavailability of therapeutic protein [18].

There have been reports of prolonged penetration and permeation-enhancing properties of Polyelectrolyte complex nanoparticles (NPs) for the delivery of peptide drugs to oral cavity in mucoadhesive delivery system [19].

The fast clearance of some drug formulations from gastrointestinal tract inhibits well absorption of them.

Some drugs remain attached to the mucous membrane for a longer period because of their mucoadhesive nature, which protects drug from the mucociliary clearance system and subsequently increases the bioavailability of the drug.

Absorption of drug via the mucous membranes is faster because of its high permeability [18].

There are many determining factor for effective delivery of intact protein/peptide through the gastrointestinal tract including the interaction between tissue surface and drug and the residence time. So, implementation of adhesive in drug delivery system might surge residence time of drug in particular in oral cavity [20]. In order to enhance the effectiveness of mucoadhesive carriers their structure could be modified.

In a research report, graft co-polymer networks was utilized as a promising platform to circumvent barriers of oral protein delivery. In this regard, Marks and co-workers designed hydrogels with and without PEG tethers and different aldehyde-modified PEG percentages (0.06%, 0.6% and 3.3%) for improvement of adhesive capacity to small intestine. The obtained results are expressed that increase of PEG percentage in the modified formulation led to high protein release percentage, approximately 80%. This phenomena could be attributed to attach aldehyde to the amines of glycoproteins in the mucous layer, providing PEG more viable for efficient proteins/peptides delivery [21].

Makhlof and et al evaluated capability of the some permeation enhancers for the mucoadhesive nanoparticles. Interaction of spermine and polyacrylic acid lead to form polyelectrolyte complexes. Fluoroscein isothiocyanate dextran was applied to investigate the permeation enhancement in caco-2 cell line and Male Wistar rats conditions. According to the visualized images by confocal microscopy, a strong as well as prolonged penetration was illustrated from fluoroscein isothiocyanate dextran-loaded with spermine-polyacrylic acid nanoparticles (about 5.56-folds), in comparison with pure fluoroscein isothiocyanate dextran [22]. Caco-2 cell lines were selected to evaluate the cytotoxicity of delivery system at permeation enhancement concentration . In aforementioned study, flouresceine-labeled polymer not only was useful to demonstrate the strong relationship of the polyelectrolyte complex to the cell lines, but also was efficient for the calcitonin oral delivery in the treated rats with aim of significant reduction in blood calcemia. Overall, the obtained data from this study revealed that calcitonin incorporated in spermine-polyacrylic acid nanoparticles possessed improved hypocalcemic effects rather than spermine solution, free drug, and Mg-polyacrylic acid nanoparticles[22].

Distribution of thiolated mucoadhesive polyacrylic acid and chitosan nanoparticles on intestinal mucosa was examined by Dunnhaupta et al. Modification of these nanoparticles was occurred by their conjugation with cysteine and 2-iminothiolane via ionic gelation and fluorescent Alexa Fluor 488 dye labeling and fluorescein diacetate strategy. Diffusion manner for both

unmodified and modified polymeric nanoparticles was evaluated *in vitro* conditions using natural porcine intestinal mucus. In comparison to unmodified, modified nanoparticles displayed a considerable increase (about 6-fold) in mucoadhesive properties. The obtained data suggested that thiolated nanoparticles mucoadhesion is more than the diffusion into the gut mucosa [23].

In a similar study, it investigated mucoadhesive property of nanoparticles by trimethyl chitosan–cysteine conjugate, which fabricated through self-assembly approach. Indeed, employment of polymeric conjugations in this research displayed a suitable mucoadhesive activity and subsequently enhancement in the insulin transport rather than unconjugated formulations owing to the formation of disulfide bonds between the mucin and trimethyl chitosan. Furthermore, high biocompatibility of conjugated formulations has been provided a significant increase in insulin internalization suggests them as a safe oral delivery system with improved permeability and mucoahesion features [24].

Quaternization of nanoparticles is another appropriate strategy for design of the effective peptide/protein delivery systems [25]. In this case, it has been reported that quaternized amphiphilic polyallylamine polymers possess the protective role against proteolytic enzymes with high complexation efficiency, nearly 78–93%. Besides, these quaternized structures are supplied low cytotoxicity in comparison to naked polymers [26].

3. CONCLUSION AND FUTURE PERSPECTIVE

Recently, there has been a significant progress in the development of noninvasive delivery systems for biopharmaceuticals. Oral delivery is the far most preferred route due to related advantages such as easy administration and high patient compliance. Due to poor plasma concentration of therapeutic peptides and proteins, oral delivery of them has always been an important challenge for researchers. Nevertheless, there are only a few formulations of oral peptide and protein in the market in recent years but the ongoing investigation in this field promises a novel avenue that can support the efficient administration without any physical and chemical modification of active component. Mucoadhesion-based nanomaterials are focused more to improve the drug absorption by bonding to the GI tract' mucosal layers . However, penetration efficiency is increased with this method but the drug protection from low pH environment and the proteases is still a problem that needs imperative consideration. The polymeric nanoparticles based delivery systems preserve the therapeutic peptide molecule from proteases and often are based on pH-sensitive release so that peptide is released in an environment with neutral pH and protected from degradation. In conclusion, currently, due to progress of oral delivery systems, in the near future numerous therapeutic proteins/peptides can be administered orally. Moreover, there are several factors such as toxicity, individual differences, quality of protein, targeting delivery to a specific site that is necessary to be addressed. Among all available delivery systems, it is difficult to narrow down them to a few delivery approaches that contain all improved pharmacokinetic properties of the peptides/proteins. Therefore, combination of the all aforementioned factors in a single drug delivery system needs to be investigated with efficient effects and commercial viability .

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