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Peroral administration of peptides and proteins

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ABSTRACT

In recent studies, parental product remains the most crucial means for administering of the therapeutic agent with proteins and peptide due to its poor bioavailability. Therapeutic proteins are becoming more important in an ever-increasing part of the healthcare system. The structural and therapeutic property of these molecules makes them more dependable on drug delivery technology so that they can achieve their maximum effectiveness. However, formulating proteins such that they maintain their stability and that they are delivered within their efficacious and safe target doses remains a challenge. The protein delivery systems reviewed in this article have been divided in to three groups, the oral, intestinal and colonic deliver

Introduction

Resurgence is underway in the bulk peptide market, with many bulk peptides producers, reporting industry growth in double digits. Discovery of new peptide molecules, improved formulation, delivery systems, and opportunities in the generic market are contributing to this growth. As therapeutic ingredient "peptides are generally non-toxic, have few side effects and represent the best avenue of therapy for many diseases, when they can be successfully delivered to the target tissue". The high potency of peptides translates into small dosage requirements [1].

Historically, peptide and protein pharmaceuticals have been delivered by paranteral administration because they are neutralized after oral intake due to their sensitivity to acidic conditions and enzymatic digestion. Some of these peptides can be given by transdermal and nasal delivery systems. However, it is still a challenge to deliver proteins and peptides orally into the systemic circulation. The drawback such as poor absorption due to bulky size and high hydrophilicity prevent designing oral dosage forms. Chemical modification of the polypeptide can increase absorption and render it less instable and perhaps increase its lipophilicity [2]. However, it is not possible to modify the structure of several peptides whose activities are dependent on tertiary structure and steric confirmation. Although the cyclic polypeptide such as cyclosporine, found to be well absorbed from the GI tract [3]. Several attempts made to improve their bio-availability following oral administration failed due to lack of established, scientific concepts[4]. Colloidal delivery systems like liposomes, microsphere, or emulsion system can protect peptides and proteins from the harsh condition of the GI tract. Addition of surfactant could greatly increase the membrane permeability however; clinical studies have not confirmed these concepts [5-7]. The major biological barrier to the oral delivery of peptide-based drugs includes the intestinal lumen, intestinal mucosa, and biochemical barrier. This review will focus on the formulation strategies used to enhance oral bioavailability of peptide based drugs. The drug polymer conjugate created provides molecular stability, transcellular transport and better pharmacokinetics. Depending on the pharmacodynamics of the peptides, various oral mucosal delivery systems can be designed. However, the physico-chemical and biological properties of these agents impose limitations in formulation, and development of optimum drug delivery systems as well as on the route of delivery.

Oral Cavity

Over a decade, there has been a particular interest in delivering drugs, especially peptides and proteins via the buccal route. Peptide absorption occurs across oral mucosa by passive diffusion and it is unlikely that there is a carrier mediated transport mechanism [8], and it provides direct entry into the systemic circulation thus avoiding first pass effect and degradation in GIT. Lozenges and sublingual tablets have been used for several decades. Based on the buccal absorption test by Beckett and Triggs [9], it was found that the buccal absorption of drugs is significantly correlated with their pH dependent renal excretion and protein binding [10]. Studies have shown that the rate of drug appearance in the systemic circulation through the buccal mucosa is slower than the rate of drug disappearance from the buccal cavity [11]. These findings have confirmed by others for meperidine [12], morphine [13-14] and Insulin [15-17]. Other than the low flux associated with buccal mucosal delivery, a major limitation of the buccal route of administration is the lack of dosage form retention at the site of absorption. However, adhesive dosage forms such as gels, films, tablets and patches can overcome these limitations. They can localize the formulation and improve the contact with the mucosal surface to improve absorption of peptides and proteins [18]. Although several studies have examined factors influencing systemic oral

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mucosal delivery of small molecular weight compounds and peptides, there are very less data available on local administration of drugs to the oral epithelium. The transforming growth factor beta (TGF- β3) diluted in three different vehicles were applied to the tissue samples mounted in perfusion cells maintained at 37°C, have shown that ^{125}I TGF-β3 was relatively stable in saliva and in the epithelium. Penetration of ^{125}I TGF-β3 to the basal layers was concentration dependant. The data suggest that the topical application of TGF- β3 to the oral mucosa in an appropriate vehicle can provide effective therapeutic delivery to the tissue [19-20]. Chitosan, a mucopolysaccharide, has been claimed to act both as a bioadhessive and permeabilizer. Permeability enhancement effect of chitosan in gel form for oral mucosa was investigated with transforming growth factor – beta (TGF-beta). Permeability was determined by measuring the flux of TGF-beta across porcine oral mucosa in an *invitro* system. It was found to exert a marked permeabilizing effect on buccal mucosa. It has been shown that buccal penetration can be improved by using various classes of transmucosal and transdermal penetration enhancers such as bile salts, surfactants, fatty acids and their derivatives, chelators, cyclodextrin and chitosan. Among these chemicals used for drug permeation enhancement, bile salts are most common. The enhancing effect of chitosan on buccal permeation of hydrocortisone and transforming growth factor beta (TGF-β) has been reported [21]. Several Transmucosal Therapeutic Systems (TmTs) were developed to study the enhanced/controlled delivery of luetinizing hormone-releasing hormone (LHRH) through oral mucosa for prolonged periods. Nakane et al performed transmucosal permeation kinetics of LHRH delivered by the various TmTs formulations containing a stabilizer, cetylpyridinum chloride, and a permeation enhancer, such as bilesalts, to enhance the stability and permeability of LHRH [22]. The results indicated that TmTs are relatively safe and capable of achieving enhanced and controlled transmucosal delivery of peptide drugs. Instead of bioadhesive tablet and patch, gel formulation with pluronic F-127 was developed for buccal delivery of insulin. Findings demonstrated that 20% pluronic F-127 gel containing unsaturated fatty acids is a potential formulation for the buccal delivery of insulin. Flat faced core tablet containing 12 or 32mg of hakea (mucoadhesive component) and 40mcg (200 IU) of Salmon calcitonin (sCT) per tablet was formulated by direct compression. Serum calcium concentration indicated that sCT was delivered across the rabbit buccal mucosa [23]. A biocompatible mucoadhesive buccal patch of peptide Oxytocin (OT) was prepared by carbopol 974 P and silicone polymer. Plasma OT concentration of loaded mucoadhesive patches remained 20 to 28 fold greater during 0.5 to 3.0 hours than control animals administered placebo patches. Protein binding, oral delivery of these therapeutic drugs enhances the value of these agents to impart its pharmacological active in an effective manner to the target site.

Intestinal delivery

The normal structure and function of the GI epithelium are generated, by the action of over 20 different peptide and small molecular weight (MW) proteins such as transforming growth factors, trefoil peptides epidermal growth factor, and pancreatic secretary trypsin inhibitor [24]. Advances in biotechnology have lead to the availability of some synthetic and recombinant forms of peptides and proteins. However, the stress conditions in the GIT, such as the low pH in the stomach and the proteolytic activities in both the stomach and the small intestine, may lead to this early and irreversible inactivation [25-27].

In general, poor absorption of peptides across mucosal surfaces is caused by the high polarity and high molecular weight of this class of compounds and their susceptibility to proteolytic degradation both by brush border and cytosolic enzymes. Intestinal peptide absorption is further more reduced by the hostile environment of the gastro-intestinal tract i.e. the strong pH extremes and the abundant presence of very potent luminal enzyme systems [28]. On the other hand, peptide absorption in the duodenal intestinal part may show some advantageous features due to the minor brush border and cytosolic enzyme activity in comparison to the jejunal and ileal parts, and due to the large surface area of the upper intestine part for rapid peptide absorption in comparison to the colon [29-30]. Different drug carrier systems have been studied to deliver the peptide pharmaceuticals per-orally like Mucoadhesive polymers, Polymeric nanoparticles, Microspheres, Liposomes etc.

Mucoadhesive polymer

One main parameter for the poor bioavailability of therapeutic peptides and proteins is presence of intestinal luminal enzymes. Attempts made to reduce the presystemic metabolism include prodrugs [31-33], Polymeric nanoparticles [34-35], Microspheres [36-37], and Liposomes. Muco-adhesive polymers such as polycarbophil and carbomer per se display an inhibitory effect on the hydrolytic activity of trypsin, alpha chymotrypsin and carboxypeptidase. A number of elastatinal polymer conjugates were synthesized and their protective effects from enzymatic degradation caused by elastase as well as their mucoadhesive properties were evaluated [38-40]. An enzyme assay determined the protective properties of modified as well as unmodified polymer. In another study the covalent attachment of EDTA to the primary amino groups of chitosan excelled conjugates. The conjugates were isolated by exhaustive dialysis against dematerialized water and tensile studies carried out on native porcine mucosa. Tensile studies revealed the enhanced mucoadhesive properties of conjugate having ratio of EDTA 1:20 and 1:40 compared to chitosan Hcl [41]. The binding affinity of the tested chitosan – EDTA conjugate towards calcium and zinc was much higher than that of poly (acrylate) derivatives. The high binding affinity found for the chitosan-EDTA conjugate may explain its strong inhibitory effect, according to the theory that serine proteases can be inhibited due to the depletion of Ca^{2+-} ions [42-45]. In order to generate chitosan-derivative exhibiting even higher complexing properties than the chitosan-EDTA conjugate, diethylene triaminopenta acetic acid (DTPA) displaying a relative higher association constant towards zinc than EDTA was prepared. Chitosan, the cationic polymer and chitosan-EDTA, an anionic polymer, exhibit significantly higher detachment force than the cationic as well as anionic polymers of chitosan DTPA. Mucoadhesion of cationic polymers seems to be based on electrostatic interaction with negatively charged moieties of the mucus [46] on the other hand, for anionic polymers such as chitosan-EDTA; muco-adhesion can be explained by the hydrogen bond formation of their carboxylic acid groups with the mucus gel layer. Chitosan derivatives exhibit a better solubility stronger muco-adhesive capabilities and enzyme inhibitory property. This unique feature makes chitosan and in particular its derivatives a valuable excipients for the per oral administration of peptide drugs [47].

Nanoparticles

The concept of solid nanoparticles was proposed and pioneered by Speiser and coworkers. Polymeric nanoparticles (NP 10-1000nm) allow encapsulation of the drugs inside a polymeric matrix protecting them against enzymatic and hydrolytic degradation. It was shown that oral application of NP

containing insulin reduced blood glucose levels in diabetic rats for upto 14 days [48]. One limitation of NP as oral delivery system is the requirement that particles need to be absorbed from the gastrointestinal tract at a sufficient rate and extent. One strategy to overcome the gastrointestinal barrier is the association of the drug with a synthetic colloidal carrier system. This concept provides improved drug stability against enzymatic degradation in the harsh intestinal environment due to the protection offered by polymer matrix [49]. Protein and antigen can be encapsulated into the NP and/ or adsorbed to the surface by physical or chemical mechanisms. Formulation of colloidal polymeric carriers is often anything but straightforward. Oppenheim et al first attempted to prepare an oral colloidal system with pure insulin without polymer. The specificity of the particles used was that they consist only of cross-linked insulin. The study was carried out on mice and rats. The absorbed insulin remained biologically active, since in some animals, the blood glucose concentration could be reduced to about 15 to 20% of the starting level. However, higher doses of nanoparticles needed to preclude the development of a commercially viable product. The oral administration of insulin using particulate system has been fairly successful in the case of rodents and dogs. Moreover, doses required for oral administration are extremely high compared to parentral doses. A new biodegradable polymer nanoparticles of poly (ethylene glycol) (PEG) coated poly (lactic acid) (PLA) nano particles, chitosan (CS) coated poly (lactic acid glycolic acid) (PLGA) nanoparticles and chitosan (CS) nanoparticles have been tested for the ability to load protein to deliver them in an active form, and to transport them across the nasal and intestinal mucosa. Nanoparticles having mean size of 196nm and negative zeta potential shown encapsulation efficiency of only 35% due to the partition of the protein between the inner and external aqueous phases[50]. The stability of these nanoparticles in simulated physiological fluids has been studied. In vitro results describing the stability of the nanoparticles in the gastrointestinal fluid revealed that the PEG coating has a role in preventing the enzyme-mediated aggregation typically observed for PLA nanoparticles. Lowe and Temple prepared calcitonin loaded polyacrylamide nanospheres by polymerization in an inverse microemulsion. The nanospheres obtained with this technique had a size below 50 nm, but the loading efficiency remained low (< 5%) and peptide was immediately released from the particles after rehydration, thus impeding the protection against protease degradation [51]. In the case of the w/o/w double emulsion technique, for the encapsulation of proteins, homogenization using ultra-sonication or high-speed turbines must be employed to obtain dispersions in the required size range. Many proteins are sensitive to high shear stress and are destroyed during NP preparation. The localization of the proteins after preparation is also often unknown. Therefore, novel methods based on solvent displacement and salting out have received increasing importance [52], because they provide less stress to protein drugs. A systemic modification of polymers in order to optimize them for NP process has not been performed.

A retrospective analysis of literature data concerning Vranckx *et al*[55] patented a preparation method, leading to the formulation of nanocapsules that have a complete inner aqueous core, facilitating the incorporation of hydrophilic compounds. The final product containing calcitonin was a suspension of nanocapsule in Mygliol®, which is an excipient that is acceptable for oral administration [53]. The chemical stability of calcitonin was at least 94% after one year of storage at 4° C. Proteolytic degradation of human calcitonin and insulin in poly isobutyl cyanoacrylate nanocapsules was slower than the free peptides in solution and polyacrylamide nanoparticles were produced using water in oil emulsion system. The calcitonin nanocapsules administered duodenally to rats at 0.2mg/kg and compared with a control of the same dose of calcitonin dissolved in 0.1% acetic acid gave a later t_{max} at 15-30min compared with the control, which had a t_{max} of 5min. The pharmacokinetics reflected the degradation resistance of the nanocapsules, generating profiles characteristic of sustained delivery. Although nanocapsules would act as depot formulation for parentaral use as suggested by Couvreur *et al* [54], they are not likely to be feasible system for the oral delivery. The current hypothesis to explain the uptake of small amounts of particles is that there is a selective absorption via M cells especially in Payer's patches. Particles that dissolve above a given pH value are potentially interesting for releasing the peptides at a specific site of the GIT. The physiochemical characteristic of Insulin is not ideal for preparation of nanoparticles. Novel drug polymer conjugate that forms its own nanoparticulate delivery system, which was named as co-polymerized peptide particles (CPP) system by the authors. A derivative of LHRH, a deca peptide i.e. vinyl acetate derivative of LHRH was first prepared and then copolymerized with butyl cyanoacrylate. The co-polymer particles were found to be stable in vitro when incubated for 3 hr in gut luminal contents and mucosal scrapings. Although the half-life of LHRH in blood is normally 2-8 min, dosing with the CPP system allowed detection of the peptide for a prolonged period of 12h. This confirmed the suitability of the CPP to promote oral uptake of LHRH [55].

Although uptake of NP through the gut wall by different mechanisms seems to be uncontested, the usefulness of this phenomenon to increase oral bioavailability of macromolecule hydrophilic drugs remains to be demonstrated. Much work remains to be achieved before polymer particles can be seen as a viable concept for the oral administration of peptides. These studies offer an innovative strategy for the oral delivery of peptides and proteins normally not absorbed through the intestine.

Furthermore prolonged contact of NP with absorptive gastrointestinal cells may be achieved using bioadhesive polymer. Various colloidal carrier systems have been studied for absorption enhancement of peptides, such as sub-micron emulsion, lipid suspensions, liposomes [56-57], polymeric nano-microparticles [58-59].

Microspheres

Polymer microspheres can also be used to deliver proteins. Microparticles made of bio-erodible polymers offer the possibility of creating tailor-made system for controlled release. Double emulsion technique can be used to prepare microcapsules and size of capsules can be altered based on the viscosity of the solution and on the velocity of the mixing processes. Additives like PEG 6000 reduced the encapsulation of a model peptide whereas sodium sulfate increased the encapsulation efficiency. The pH of the organic phase has an important impact on the encapsulation. The influences of pH, polarity and viscosity of the surfactant phase on encapsulation still have to be determined. Small thermoplastic microspheres delivered orally are able to cross the intestinal epithelium, either through the Payers patch [60-61] and/or the regular absorptive epithelium [62]. Three major mechanisms for improving oral delivery of proteins first by the spheres can protect proteins from proteolysis, second the spheres cross the intestinal mucosa and third the microsphere change the inter tissular distribution of the protein throughout the organism.

In a specific study, the administration of 100 IU/kg of insulin in 250-300nm isobutyl 2-cyanoacrylate microspheres directly into the duodenum, jejunum, ileum and colon led to reduction in serum glucose levels. The greatest blood glucose reductions of 65% was seen in the ileum while a 50% reduction was seen in the duodenum, and jejunum and a 30% reduction was seen in the colon [63]. In an earlier study, a similar oral insulin formulation of 25 IU/kg insulin in isobutyl 2-cyanoacrylate microspheres produced 50% reduction in fasted serum glucose levels in streptozotocin induced diabetic rats as compared to 60% reduction in rats given the same dose subcutaneously [64]. The pH sensitive micro-particles can be prepared using derivatised amino acids [65]. Benzoylated and phenyl sulfonylated single amino acids are novel, low molecular weight, and self-assembling in nature. At low pH, these molecules aggregate to form microspheres that will dissolve itself readily under neutral conditions. With this technique, entrapment of nearly 60% dissolved peptides was reached. Morishita *et al* [66] investigated the possibility of preparing pH sensitive particles that would dissolve in the jejunum or the ileum. To enhance the potentials of these particles, they added a protease inhibitor (aprotinin). Recent experiments revealed that the mucoadhesive polymer, polycarbophill, can virtually able to improve the intestinal absorption of 9-desglycinamide-8-arginine vasopressin (DGAVP). A controlled release bioadhesive drug delivery system was tested, consisting of microspheres of poly (2-hydroxyethyl methacrylate) with a mucoadhesive polycarbophil-coating, as well as fast release formulation consisting of an aqueous solution of the peptide in a suspension of polycarbophil particle [67]. The effect appeared to be dose-dependent, indication of intrinsic penetration enhancing properties of the mucoadhesive polymer. A prolongation of the absorption phase in vitro in the chronically isolated loop in-situ suggested that the polymer was able to protect the peptide from proteolytic degradation. Luessen et al [68] studied the effect of the polymer on trypsin activity by measuring the degradation of a trypsin specific substrate. Trypsin inhibition was found to be time dependent upon addition of Ca^{2+} in the degradation experiment. Circular dichroism studies suggested that under depletion of Ca^{2+} from trypsin, the secondary structure changed its confirmation, followed by an increased auto degradation of the enzyme Carbomer, which had a high inhibitory effect on trypsin activity and also having high Ca^{2+} binding affinity than polycarbophil [69].

Liposome

The potential usefulness of liposomes as drug carriers has attracted considerable interest [70]. The drugs encapsulated in liposomes are sufficiently protected from enzymatic attack and immune recognition. Various attempts have been made to apply liposomes to the preparation of oral insulin [71]. Phospholipid vesicles are capable of encapsulating both hydrophobic and hydrophilic drugs they are biodegradable and are non-toxic. Dipalmitoyl phosphatidyl choline and dipalmitoyl phosphotidyl ethanol are studied for their resistance to pancreatic phospholipase A2 catalyzed hydrolysis. The entrapment of insulin increased with a rise of proportion of negatively charged phosphatidyl ethanol in the mixture [72]. The study suggests that lipid composition and physical state of liposomes were important determinants of therapeutic effect. The acetated tryptic digest insulin made by negatively charged liposome containing phosphatidyl inositol, which enter the blood stream, and will be competitive in receptor binding with hormone molecules [73].

Colonic delivery of peptides and proteins

Colonic drug delivery is a relatively new scientific area that has been developed during the last 10 to 15 years. The experience gained during this period is of great importance for the development of targeted delivery system with reliable drug release property. A reliable colon drug delivery could also be an important stern position for the colonic absorption of per orally administered undigested; unchanged and biologically active peptide drugs [74-75]. Since the large intestine is relatively, free of peptidases [77].

Different strategies for colon specific delivery of peptides and proteins

Pro drug strategy

A pro drug is a pharmacologically inactive derivative of a parent compound that requires spontaneous or enzymatic transformation within the body in order to release the active drug. Colon targeted pro drug design exploits bacterial enzymes specific to the colon. The main enzymes produced by bacterial flora are azoreductase, glycosidase and cyclodextrinase. The main pro drugs are azo bond pro drugs and glycoside pro drugs. Unfortunately, the synthesis of peptide pro drugs has been limited due to their structural complexity and the lack of methodology for their efficient synthesis [78-79]. Kahns et al [80] has prepared several pivalate esters of desmopressin, a synthetic analog of the antidiuretic hormone, vasopressin. These derivatives were converted quantitatively to desmopressin by enzymatic hydrolysis in human plasma. The transport of the pivalate pro drug across monolayers of CaCo2 cells was found remarkably higher relative to desmopressin. Bioreversible cyclization of the peptide backbone is one of the most promising and intriguing new approaches in the development of peptide pro drugs. Cyclization of the peptide backbone enhances the extent of intramolecular hydrogen bonding and reduces the potential for intermolecular hydrogen bonding to aqueous solvent. In addition, cyclization of the backbone blocks the C terminal carboxyl group and the N terminal amino group, so that the peptide is less susceptible to enzymatic degradation mediated by amino and carboxy peptidases. Pauletti et al. [81] prepared cyclic, esterase- sensitive pro drugs of a model peptide, H-Trp-Ala-Gly-Gly-Asp-Ala-OH. These pro drug systems were prepared by linking the N terminal amino group to the C terminal carboxyl group via an acyloxy alkoxy promoiety. The metabolic stability in biologic milieu was substantially greater when compared to that of linear hexapeptide and transport studies across monolayer of CaCo2 cell have shown 70 times more permeation compared to that of linear hexapeptide. Based on the results obtained by Conradi et al [82], a reduction in the number of potential hydrogen bonding sites may increase the permeation characteristics of peptides across the intestinal mucosa. The coumarinic acid based cyclic pro drugs were designed to release (Leu) enkaphalin and its metabolic analog DADLE [H-Try-D-Ala-Gly-Phe-D-Leu-OH] by enzymatic hydrolysis of the ester promoiety to form intermediate that would then under go lactonization to release the parent peptide [83]. When [Leu] enkephalin was applied to the apical side of Caco-2 cell monolayers, an *invitro* model of the intestinal mucosa, it degraded rapidly (t1/2 = 15 min) [84]. In contrast, DADLE was substantially more stable. Like the coumarinic acid based cyclic pro drugs described above, phenyl propionic acid based cyclic pro drugs were designed to release [Leu] enkephalin and DADLE. Unlike the coumarinic acid based cyclic pro drugs, the lactonization reaction is very fast and that the rate-determining step is the hydrolysis of the phenolic ester. In a biological milieu, the phenyl propionic acid based cyclic pro

drugs were expected to degrade faster to the opioid peptides because of the presence of esterases. It is interesting to compare the stability data in biological medium for the phenyl propionic acid based cyclic pro drug. The stability data for their coumarinic acid based counter parts. The observation that the coumarinic acid based cyclic prodrugs of [Leu] enkaphalin and DADLE were significantly unstable in biological media than were the phenyl propionic acid based cyclic pro drugs [85]. The result of these studies suggest that cyclization of linear peptides could have a dramatic effect on their metabolic stability and their ability to permeat across biological barriers. Azo polymers comprise of both aliphatic and aromatic polymer, having the azo functionality. Aliphatic azo polymers are thermally unstable and decompose into free radicals, a property, which is frequently used in the initiation step of free radical polymerization. It was recognized that aromatic azo polymers could have a potential for colon targeting since reduction and subsequent splitting of the azo bond only occurs in the large intestine. The metabolism of azo compounds by colonic bacteria is one of the most extensively studied bacterial metabolism processes. It is suggested that both an intracellular enzymatic component and extracellular reduction exist [86].

Azopolymer coated tiny compressed tablets containing vasopressin were tested by oral administration to hydrated rats in an apparatus that automatically recorded urine formation. After a delay generated by the transit of the dosage form through the gastrointestinal tract, urine formation decreased abruptly as an indication of vasopressin load [87]. In another study vasopressin dosage form was prepared by deposition of a vasopressin solution on tiny slivers of filter paper, and coated with azopolymers. Similar dosage form containing 1 unit each of insulin were prepared and given orally to rats. The insulin provoked decrease in blood glucose levels of rats made diabetic with streptozotocin [88]. Molecular modeling of low molecular weight azo compounds revealed that reduction of azo bond to hydrazo intermediate requires a low electron density within the azo region, and thus substitution of electron-withdrawing groups will favour this reaction. Co polymers of styrene and 2- hydroxy ethyl methacrylate were synthesized in the presence of 4-4'-divinylazobenzene or N, N'-bis (β-styryl sulfonyl)-4-4'-diamino benzene to develop biodegradable coatings. Oral administration of azo polymer coated vasopressin and insulin to rats produced biological responses characteristic of the peptide hormones. However, problems related to variability in absorption rates were encountered which were probably due to inter, and intra subject differences in microbial degradation of the coatings. In a study with dogs, azo polymer coated capsules delivered insulin after oral administration, but there was no clear dose response relationship between the amount of administered insulin (single dose) and the portal concentration of insulin [89]. To facilitate absorption of insulin from the larger colon, the absorption enhancer, 5-methoxy salicylates was added to the capsules. In this study, dogs were made diabetic by pancreatectomy. Cannulae and Doppler flow probes were placed in the hepatic portal vein and hepatic vein, to permit samples of blood entering the liver from the intestine and leaving the liver to be taken. Capsules of insulin (50-150 unit/dose) and 5 methoxy salicylates (15-18mg/dose) coated with azo polymer were given to the dogs. Evidence for the absorption of insulin from the intestine was obtained by a sudden rise in the insulin content of plasma from the cannula in the hepatic portal vein. However, after a single dose, the decrease in glucose was small and did not last long.

Novel azopolymer coated pellets containing insulin was developed by Tozaki et al [90]. The intestinal absorption of insulin and eel calcitonin after oral administration of the azopolymer coated pellets containing these peptides with camostate mesilate was evaluated by measuring the hypoglycemic and hypocalcemic effects. The findings suggest that azopolymer coated pellets may be useful for colon specific delivery of proteins including insulin and Asueel calcitonin. The extremely large variation in glucose levels after administration of insulin with azo polymer coated capsule to beagle dogs could also be explained in terms of difference in GI transit and microbial degradation in different dogs [91]. To rationalize partially the differences encountered in microbial degradation of azo polymers, Van den Mooter and co-workers [92] studied the influence of polymer hydrophilicity. However, a balance between the hydrophobic and hydrophilic constituents of the polymer is important in optimizing the system. Incorporation of methacrylic acid in the polymer backbone yielded water insoluble polymers with a pH dependent degree of swelling. In vitro and in vivo studies showed that polymers with the highest degree of swelling in the colonic environment were degraded faster [93]. Depending on the nature of the polymer, the azo reduction can result in complete chain cleavage (formation of amines) or can stop at the intermediate stage (hydrazoformation).

Transport of Lau-TRH across intestinal membrane was much higher than that of free TRH or TRH with diff erent absorption enhancers This increased peptide uptake was due to non-specific c binding of Lau-TRH to brush-border membrane and involvement of the ligopeptide transporter in the small intestine. Prodrugs of TRH that are characterized by higher lipophilicity than TRH were patented by Bundgaard and Moss who claimed they possess a higher resistance toward degradation by TRH-inactivating enzymes. The relatively recent evolution of recombinant DNA research and modem synthetic methodologies allows the biochemist and chemist to produce vast quantities of various peptides and proteins possessing pharmacological efficacy. However, the therapeutic potential of these compounds lies in our ability to design and achieve effective and, stable delivery systems. The future challenge in biotechnology may not only be polypeptide cloning and synthesis but effective nonparenteral delivery of intact peptides and proteins to the systemic circulation and their site of action. Based on our current understanding of biochemical and physiological aspects of peptide and protein absorptiona and metabolism it is difficult to conceive of efficient means of delivering of these agents through the use of conventional formulation technology, namely, simple tablets and capsules.

pH sensitive polymer

A number of commercially available methacrylic resins, popularly known as Eudragits, are being used for colon-targeted formulation. Polymer with nonesterified phthalic acid groups dissolve much faster and at a lower pH than those with acrylic or methacrylic acid groups.

A series of N- acylated alpha amino acids were synthesized and shown to improve the oral delivery of protein drugs. Histological examination of rat intestinal tissue after oral dosing of acylated amino acid/protein combination revealed no detectable pathology [94]. Polymeric beads prepared from pH/temperature sensitive linear terpolymer (poly N- isopropyl acrylamide – co- butyl methacrylate – co-acrylic acid) were loaded with human calcitonin. The quantity and the physical state of the peptide in the formulation were analyzed by reverse phase HPLC, CD and FTIR. The loading and stability of

calcitonin improved significantly as the acrylic acid content increased from 0 to 10-mol% [95]. Another study suggested that polyacrylic acid polymers can inhibit luminal degradation of insulin, calcitonin and insulin like growth factor I (IGF-I) by trypsin and chymotrypsin. Polymers at 1% and 4% (w/v) inhibited close to 100% of trypsin and chymotrypsine acting against insulin. Studies revealed that the inhibitory effects of carbopol polymers correlated with the final pH in the incubation medium that has no or less buffer capacity to buffer the proteins released by carbopol polymers. These polymers are able to reduce the pH much lower than the optimum pH for the enzyme activity, and thus inhibit proteolytic degradation [96]. The influence of pH variability through the stomach to the intestine on the oral bioavailability of peptide and protein drugs may be overcome by protecting them from proteolytic degradation in the stomach and upper portion of the small intestine using pH-responsive hydrogels as oral delivery vehicles. Lowman et al., for example, loaded insulin into polymeric microspheres of poly (methacrylic-*g*-ethylene glycol) and observed oral bioavailability in healthy and diabetic rats. In the acidic environment of the stomach, the gels were unswollen as a result of the formation of intermolecular polymer complexes. The insulin remained in the gel and was protected from proteolytic degradation. While in the basic and neutral environments of the intestine, the complexes dissociated, which resulted in rapid gel swelling and insulin release. Within 2 hours of administration of the insulin-containing polymers, strong dose-dependent hypoglycemic effects were observed in both healthy and diabetic rats. Numerous pH-sensitive polymers have been investigated for a range of applications.

During the last decade, pharmaceutical research demonstrated the efficacy of Eudragit coating in oral selective delivery systems. A study by Morishita et al [97] compared insulin delivery by three-formulations containing Eudragit-L100, Eudragit-S100 and Eudragit LS. Formulation containing Eudragit S showed optimal delivery of insulin in the ileum at pH 7, while coating of sulfapyridin with pH sensitive Eudragit S successfully delivered more than 60% of the drug to the colon. Khan et al investigated various combinations of two methacrylic acid co-polymer Eudragit L100-55 and Eudragit S 100 by spraying from aqueous system. The coated tablets were tested in vitro for the suitability for colon targeted oral drug delivery.

Biodegradable matrices

The rational for the development of polysaccharide-based delivery systems for colon targeted drug delivery is the presence of large amount of bacterial polysaccharidases in colon. Various major approaches utilizing polysaccharides for colon specific delivery are fermentable coating of the drug core, embedding of the drug in biodegradable matrix, and formulation of drug-saccharide conjugate (prodrugs). Chitosan, pectin, chondroitin sulfate, cyclodextrin, dextron, guar gum, inulin, and amylases.

Osmotic systems independent of gastric residence time and metabolism by bacterial flora have also been developed for colon delivery. OROS-CT systems developed by Theeuwes et al [98] consist of a single or 5-6 units in one dose. These enteric-coated push- pull units contain an osmotic push compartment and a drug compartment, both surrounded by a semi permeable membrane with an orifice. As the unit enters the small intestine, the enteric coating dissolves and the osmotic push compartment containing an osmopolymer and an osmotic agent swells. Swellings of the osmotic push compartment forces the drugs get out of the orifice. These systems can be programmed to delay the drug released for varying duration.

Another strategy relies on the strong peristaltic waves in the colon that lead to a temporarily increased luminal pressure (pressure-controlled drug delivery). Pressure sensitive drug formulation releases the drug as soon as a certain pressure limit is exceeded. The pressure and the destructive force induced by peristaltic waves are certainly high in the distal part of the large intestine [99]. Pressure controlled colon delivery capsule (PCDC) having different thickness of a water insoluble polymer membrane were prepared by coating the inner surface of the gelatin capsules with ethyl cellulose. There was a good correlation between the parameters studied: EC (ethyl cellulose) coating, membrane thickness, hardness and T_i (the time when fluorescein first appeared into the systemic circulation after oral administration). The thickness of EC coating governs the release rate. A new preparation method developed by Takada et al [100] where PCDC were prepared by coating the capsule shaped suppositories with ethyl cellulose. The physical properties i.e., EC coating membrane thickness and hardness were studied. There was a good relationship between T_i and thickness. As the thickness of EC coating membrane increases T_i and MRT (mean residence time) are increased.

Solid dosage form of pectin might be used as specific drug carrier [101]. The gelling properties of pectin offers several advantages, including formation of viscous diffusion barrier and fermentation ability in the human large intestine [102] which are useful attributes in colonic drug delivery system. Two major methods used to prepare saccharidic hydrogel are: polysaccharides are reacted or mixed with synthetic polymers to create a biodegradable hydrogel and natural polysaccharides are modified to reduce the water solubility and swelling properties without affecting their ability to degrade by specific colonic enzymes. Physical mixture of methacrylic acid co polymer (Eudragit RS) and β cyclodextrin were used to prepare biodegradable films [103].

A series of water-soluble acrylic polymers, containing disaccharide side groups, were synthesized and evaluated *invitro* [104]. A cellobiose derived monomer of methacrylic acid, 4 – o - β - D glucopyranosyl-1 methacrylamido – 1 deoxy – D – glucitol, prepared and polymerized with additional molecules of methacrylic acid to produce series of co-polymers. The degradation of the disaccharides in the co-polymer was evaluated by measuring the amount of glucose cleaved upon incubation with β glycosidase. Similarly, chondroitin sulfate was cross-linked with diamino dodecane [105-107]. Pectin was precipitated with calcium chloride and guar gum was reacted with borax [108]. It was speculated that the modification would result in novel raw material for solid dosage forms that would be able to deliver, with minimal loss, at the same time they would be able to retain their ability to be degraded by the colonic enzymes. Pectins, naturally occurring non-toxic water-soluble polysaccharides, are widely used in many food industries. Amidated pectins are low methoxy pectins in which some of the carboxylic acid groups are amidated. They are more tolerant of pH variation and calcium levels than conventional pectin, which could make them useful in colonic delivery system. The swelling behavior of beads prepared with pectin is greatest at pH 5 and less swelling occur at pH 3 and 7.4. In presence of pectinolytic enzyme the beads are totally degraded. The interaction between pectin and chitosan form a complex at low pH. Since electrostatic interaction between polyanion and polycation leads to the formation of polyelectrolytic complex (PECs) which can provide a great barrier to drug release in the upper GIT than either of material alone Munjeri et al [109]. The optimum ratio of pectin to chitosan for the formation of a PEC

will vary with pH as the ionization state of the components change [110]. The addition of HPMC to the pectin/chitosan film formulation is to improve the physicochemical properties of the film such as film ductility [111-112], toughness and elasticity [113]. Such film will provide appropriate controlled release of the drug in the small intestine and the permeability properties will increase significantly when exposed to the pectinolytic enzymes present in the colon [114]. The rate of permeation through the film of pectin/ HPMC is higher than that of pectin/chitosan/HPMC film. The reduction in permeability of pectin/HPMC film is not due to the PEC formation but there may be other linkage of chitosan with pectin and HPMC [115]. Recently pectin-HPMC compression coated tablet of 5-ASA was designed by Murat and Timucin based on the gastro intestinal transit time concept. Pectin and HPMC are being hydrophilic materials; the system swells and forms a hydrogel layer when they are placed in an aqueous medium. The pure pectin coat found to be insufficient to protect the drug until 6 hr; its mechanical properties are poor because of high water solubility. Therefore, there is always a need to incorporate another polymer to produce pharmaceutically acceptable film or coating layer [116].

Guar gum an oligosaccharide consist of linear chain of 1,4 β D-mannopyranozyl units with α D galactopyranosyl units attached to (1-6) linkages [117-118]. Guar gum is commonly used in the food industry as a thickening agent. The pH of 1% w/v aqueous dispersion of guar gum varies from 5 to 7 and is stable over a wide pH ranges. Being a nonionic, the viscosity of dispersion is unaffected by pH and is same in both acidic and alkaline medium. Since a major restriction in the design of Guar gum (GG) matrices for drug delivery is its high swelling characteristic, which requires high compression force. Chemical modification of guar gum reduces its enormous swelling property. Chemical modification may done by either with borax or glutaraldehyde [119-120]. Trisodium trimetaphosphate (STMP) is a non-toxic cross linker used in the food industry to cross link starch. STMP has been used to cross link guar gums, the reaction with STMP caused guar gum to lose its non-ionic nature and the cross linking density was found to be STMP concentration dependent [121]. In vitro release study of guar gum cross linked product (GGP) containing hydrocortisone into buffer solution with or without GG degrading enzyme (α-galactosidase and β-mannanase) shown that the product was able to prevent the release of 80% of its hydrocortisone for at least 6 hrs in phosphate buffer solution of pH 6.4. When a mixture of α-galactosidase and β-mannanase was added to the buffer solution enhancement in hydrocortisone release was observed. Drug release studies of tablet formulation under condition mimicking mouth to colon transit have shown that guar gum protects the drug from being released completely in a physiological environment of stomach and small intestine [122]. Gamma-scintigraphic studies in human volunteers with technetium 99m DTPA as a tracer in sodium chloride core tablets compression coated with guar gum have shown that gum coat protect the drug in the stomach and small intestine environment [123]. It is concluded that this polymer could potentially be used as a vehicle for colon specific drug delivery.

Amylose, high molecular weight polysaccharide of starch, offers a mean of colon specificity. Amylose has been found to be resistant to the action of pancreatic enzymes within the small intestine but digested by bacterial enzymes of colonic origin [124]. Various studies have confirmed both *invitro* and in human, that amylose and ethyl cellulose coatings, after application to conventional oral dosage form, offer a reliable means of delivering drugs to the colon [125-127].

Future Prospects

During the last decade, various delivery system based on variety of strategies have been investigated for oral delivery of peptides and proteins. Although different site-specific delivery systems have been developed for delivering bioactive peptides and proteins each has got its own limits to deliver the drug. There is a substantial difficulty in targeting of peptides and proteins using currently available approaches. The prepared oral site-specific delivery systems are those, which rely on conditions encountered in the GIT, since these systems will give true site-specific delivery.

Pharmaceutical approaches such as mucoadhesive patch/tablet, coating with pH sensitive polymer, biodegradable polymer, and preparing biodegradable nanoparticles and hydrogels are currently being evaluated for oral delivery of peptides and proteins. Results published thus far show that these strategies are appealing and viable, however, no system is capable of delivering them completely and reproducibly. Recently a new design of delivery system is undergoing phase II clinical studies i.e. gastrointestinal mucoadhesive patch system. To overcome the poor membrane permeability of peptides and proteins, many scientists have been studying the use of absorption enhancers, enzyme inhibitors and enteric-coated formulation in improving the bioavailability of peptides and proteins [128-132]. However no technology has been launched yet. Recent studies have indicated that the dilution and spreading of absorption enhancer in GI tract reduce the enhancing effect of the absorption promoter [133-134]. GI-MAPS are based on patch formulation, which creates a closed space on the target site of GI mucosa by adhering to the mucosal membrane. As a result, both drugs and absorption enhancer co-exist in the closed space and high concentration gradient is formed between the system and enterocytes. Emisphere and Novartis are co-developing an oral calcitonin preparation with a protein/peptide unfolding technology. Certainly, protein-unfolding technology is a unique one and has the highest probability to develop oral protein/peptide preparation, however, the technology has some drawback such as the unfolding agent has specificity to each protein/peptide drug. However, this problem is not a critical factor for development of oral protein/peptide preparation. All the pharmaceutical scientists are expecting that protein-unfolding technology will wake up protein/peptide drug launch on to the market.

Conclusion

In conclusion, Drug delivery through oral route is challenging. The bioavailability of drugs remains to be an active area of research. Several sites in the GIT have been exploited but there are no major breakthrough with broad estimated by researchers, by studies reviewed herein indicates that several peptides and proteins can be administered orally for systemic delivery. Several strategies are currently being used to introduce them into the specific site of GIT and use of absorption enhancers, enzyme inhibitors and muco-adhesives should improve the oral bioavailability of peptides and proteins.

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