

Oral Nano-Insulin Therapy: Current Progress on Nanoparticle-Based Devices for Intestinal Epithelium-Targeted Insulin Delivery

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Abstract

Extensive research has been carried out to explore the potential ways of orally delivering insulin for the treatment of diabetes. Advancements in nanotechnology have brought the scientific community ever closer to this goal. The current review focuses on the various barriers existing in the route of oral insulin delivery and the strategies undertaken so far to overcome those by harnessing nanoparticles as potential vehicles of insulin. Additionally, an overview on the performance of various nanoparticle formulations including polymeric as well as lipid-based preparations will be given in context to their levels of success for insulin delivery through the intestinal epithelium. Finally, future prospects on nano-insulin therapy will be discussed.

Keywords: Insulin; Nanoparticles; Oral delivery; Diabetes; Chitosan; Poly(γ -Glutamic Acid); Poly(Lactide-Co-Glycolide); Poly(Acrylic Acid); Poly(Isobutylcyanoacrylate); Poly(Alkylcyanoacrylate); Poly(Ethylene Glycol); Vitamin B12; Wheat germ agglutinin; Lectin; Solid lipid nanoparticles; Tight junction

Abbreviations: NP: Nanoparticle; CS: Chitosan; γ PGA: Poly(γ -Glutamic acid); DTPA: Diethylene triamine pentaacetic acid; TMC: N-Trimethyl chitosan; CSK: CSKSSDYQC targeting ligand; Cys: Cysteine; HTCC: N-(2-hydroxyl) Propyl-3-trimethyl ammonium chitosan; PLGA: Poly(lactide-co-glycolide); PL: Phospholipid; HP55: Hydroxypropyl methylcellulose phthalate; RS: Eudragit® RS; PAA: Poly(acrylic acid); PIBCA: Poly(isobutylcyanoacrylate); PACA: Poly(alkylcyanoacrylate); MAA: Methacrylic acid; PEG: Poly(ethylene glycol); AA: Acrylic acid; P(MAA-g-PEG): Copolymer of poly(ethylene glycol)-grafted methacrylic acid; P(AA-g-PEG): Copolymer of poly(ethylene glycol)-grafted acrylic acid; PEGDMA: Poly(ethylene glycol) dimethacrylates; PLA-F127-PLA: PLA-b-Pluronic-b-PLA; PCL: Poly(ϵ -caprolactone); VB12: Vitamin B12; DPPC: Dipalmitoyl-phosphatidylcholine; WGA: Wheat germ agglutinin; TL: Tomato lectin; UEA1: Ulex Europaeus Agglutinin I; NaTC: Sodium taurocholate; SLN: Solid lipid nanoparticles; WGA-N-glut-PE: Wheat Germ Agglutinin-N-glutaryl-phosphatidylethanolamine; TJ: Tight junction; BA: Relative bioavailability; PA: Relative Pharmacological Bioavailability.

Introduction

Oral administration of insulin

Oral administration of drugs is generally preferred due to the advantages it confers as a painless, simple mode of delivery, in order to increase patient compliance to the therapy. Orally administered medications would be particularly beneficial in managing chronic diseases such as diabetes, which requires life-long therapy [1-5].

Due to the inconvenience of daily injections, insulin therapy, currently given though subcutaneous (SC) injections in diabetic patients [6-8] has led to problems with patient compliance [6,9]. Furthermore insulin administered orally enters the portal circulation and passes through the liver before reaching the systemic circulation, which closely resembles the physiological insulin secretion, as opposed to conventional SC insulin, which can lead to peripheral hyperinsulinemia and its associated complications [6,10,11].

Thus oral formulations for insulin would greatly benefit patients with diabetes requiring insulin therapy. However insulin is inherently

unstable in the gastrointestinal (GI) tract and has low permeability to cross the intestinal epithelium [6-8,12,13].

Uptake of drugs and particles in the GI tract

Particles in the GI tract can be absorbed through various sites and mechanisms. Those with a diameter of 1 μ m may be phagocytosed by intestinal macrophages, whereas smaller particles (<10 μ m) may be absorbed through the Peyer's patches in the small intestine. NPs smaller than 200 nm can be transported via endocytosis by enterocytes. Although rare and possibly dangerous, larger particles (5-150 μ m) can be taken up by villus tips by a passive process known as persorption. The factors affecting particle uptake include the particle size, surface charge or hydrophobicity, amount or concentration, as well as any incorporated targeting mechanisms on the particle that would express affinity to certain sites in the GI tract [14].

Obstacles to oral insulin delivery

The function of the GI system includes the digestion and absorption of nutrients, as well as acting as a defense barrier to pathogenic microorganisms and toxins [6,15]. As a result, numerous challenges exist for oral delivery, limiting the bioavailability of the drugs. In order to enter the bloodstream, orally administered drugs should be able to withstand the chemical and enzymatic conditions in the GI system, and be able to cross the mucus layering the enterocytes before diffusing through the intestinal epithelium (Figure 1) [6].

Extreme pH conditions and proteolytic enzymes: GI pH conditions range from the acidic gastric environment (pH 1.2 – 3.0) to the slightly basic intestinal environment (pH 6.5 – 8.0) [6,16]. This

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is particularly damaging for drugs such as insulin, a polypeptide, which would lose its activity due to pH-induced oxidation, hydrolysis or deamidation (Figure 1) [6,13]. Furthermore, protein drugs would be acted upon by proteolytic enzymes present in the GI lumen (e.g. pepsin, trypsin, chymotrypsin, elastase, carboxypeptidases) (Figure 1), as well as those associated with enterocytes (membrane-bound aminopeptidases and cytosolic proteases such as insulin-degrading enzyme) [6,17-19].

Mucus layer: In addition, drug molecules in the intestine must cross the GI mucus barrier in order to be absorbed through the epithelial layer (Figure 1). Mucus is a complex hydrogel made up of proteins (mainly mucins), carbohydrates, lipids and salts, as well as other components (e.g. antibodies, cellular debris, etc.). This semipermeable mucus layer obstructs the diffusion of protein molecules from approaching the epithelial cells. This diffusion barrier is due to the molecules in mucus

forming an obstructing mesh, the constant turnover of mucus in the GI tract, and the electrostatic repulsion between the negatively charged mucus layer and anionic protein molecules [6,20-23].

Intestinal epithelium: Intestinal epithelium also presents as a barrier to drug absorption (Figure 1). Particle size, surface charge or hydrophobicity, and the nature of the polymer used, affect the transport across epithelial layers [1,24].

Passive diffusion via the transcellular route is limited to small lipophilic drugs (molecular weight under 700 Da) [6,25]. Most protein drugs exceed 3000 Da and hence, are difficult to be absorbed this way [6,13,26]. Intercellular tight junctions (TJs) prevent most paracellular transport of drugs, with the exception of hydrophilic drug molecules weighing less than 200 Da. It is virtually impossible for proteins to be transported via the paracellular route without permeation enhancers [6,27-29].

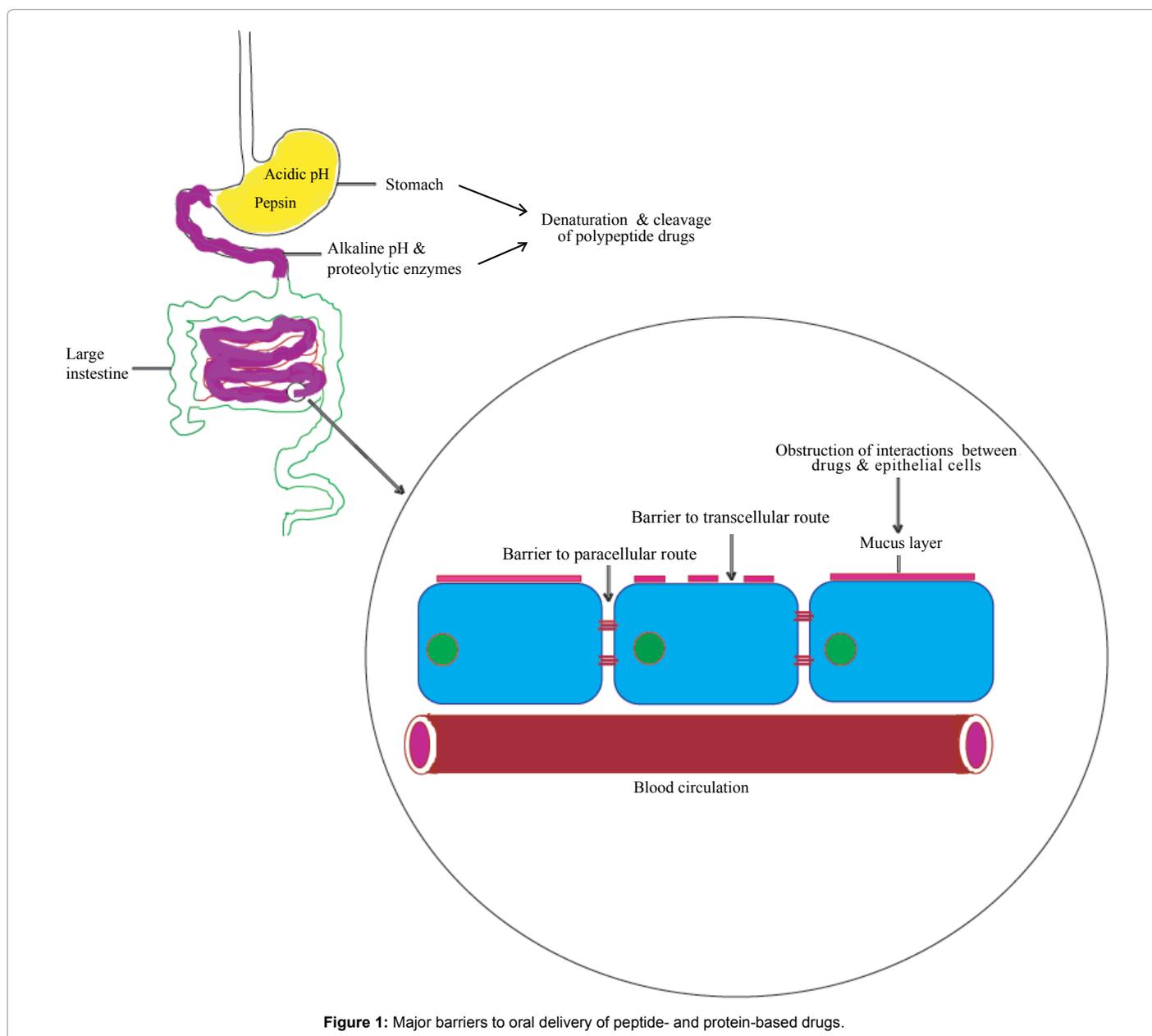


Figure 1: Major barriers to oral delivery of peptide- and protein-based drugs.

Role of nanoparticles in oral insulin therapy

Nanoparticles (NPs) are widely used as drug delivery vehicles with extensive research being made to date for harnessing the benefits of different NP formulations for oral insulin delivery (Figure 2). NPs include nanospheres (having a uniform distribution of drug within a matrix) and nanocapsules (consisting of a polymer membrane encapsulating the drug in a cavity) [1,30].

Efficient transport along GI tract: NP formulations can potentially improve the traffic of oral insulin in the GI track due to: (i) protection against pH-related and enzymatic degradation in the GI tract by encapsulation of insulin within NP carriers (Figure 2), (ii) better drug delivery close to absorption sites – via increased residence times near intestinal epithelium by mucoadhesive coating of NPs and gradual release of insulin close to the epithelial layer (Figure 2) and, (iii) improved transport through the GI mucus layer by way of mucus-penetrating particles or coatings [1,6,20,24,31].

Mucoadhesion of polymeric particles may result from one or more of the following: (i) hydrogen-bonding, (ii) electrostatic forces, (iii) hydrophobic interactions, (iv) van der Waals forces, or (v) entanglement of polymer chains with mucins [1,20,32].

Free drug molecules or drug-loaded NPs may be absorbed by the intestinal epithelium. Stable NPs made from polymers insoluble in water (e.g. PLGA) are more likely to be absorbed intact. On the other

hand, NPs composed of less stable polyelectrolyte complexes (e.g. CS) will undergo partial dissociation without being absorbed intact [1].

Therefore, the bioavailability, efficacy and specificity of drugs can be improved with the use of NPs [1,33]. However, it is vital that NPs do not cause toxicity, immune reactions, inflammation, thrombosis, and are biodegradable [1,24,30,33-35].

Facilitated transcellular transport: NPs can be taken up by the intestinal epithelial cells via different mechanisms, depending on the constituent polymers. Transcellular transport of NPs is mainly by energy-dependent endocytic processes [6,36]. These encompass phagocytosis (largely by M cells of Peyer's patches), macropinocytosis and micropinocytosis. Micropinocytosis is further divided into clathrin-mediated, caveolin-mediated, as well as clathrin- and caveolin-independent endocytosis. Among these mechanisms, phagocytosis and clathrin-mediated endocytosis are receptor-mediated [1,6].

Non-specific cellular uptake: NP uptake by cells might involve non-specific processes, which depend on particle diameter, surface charge and mucoadhesivity [6,37]. Substances exhibiting mucoadhesive properties include chitosan (CS), poly(acrylic acid) (PAA), thiomers and their derivatives, all of which are hydrophilic [6,38-41]. Adhesion and absorption of particles by intestinal epithelial cells are also increased when coated with polyvinyl alcohol (PVA) and vitamin E succinylated polyethylene glycol 1000 (Vitamin E TGSP) [6,24].

Specific cellular uptake: This occurs on NPs modified with

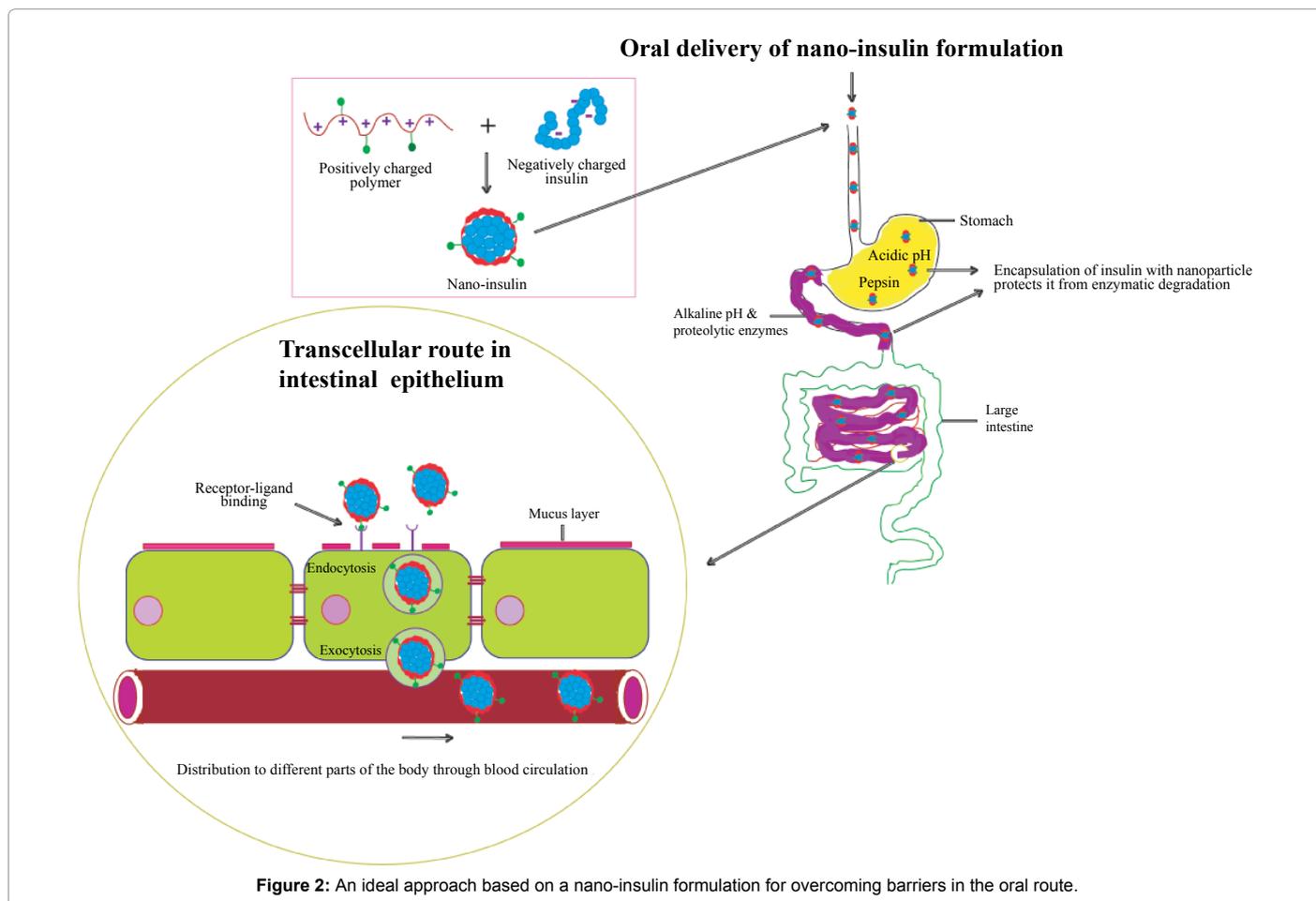


Figure 2: An ideal approach based on a nano-insulin formulation for overcoming barriers in the oral route.

ligands (e.g. vitamins or proteins) to target specific cells (Figure 2) [6]. These targeted NPs are mostly taken up by clathrin-mediated endocytosis [1,36,37,42]. For example, conjugating vitamin B12 to NPs has been shown to improve the transport of proteinaceous drugs via specific receptor recognition by enterocytes [6,43-47]. Lectins, which are proteins or glycoproteins involved in cellular recognition and adhesion, can also be conjugated to NPs to promote specific uptake [20]. Lectins have affinities to specific sugars, and lectin-modified NPs were described to have better bioadhesivity, endocytosis, and resistance to digestion [6,20,48-51]. Wheat germ agglutinin (WGA), a lectin, has been proven to bind to the surface of Caco-2 cells and enterocytes. WGA demonstrates affinity to N-acetyl-D-glucosamine and sialic acid, which are present along the intestinal tract. In addition, WGA is transported via receptor-mediated endocytosis, involving the epidermal growth factor receptor, which is widely expressed on the surface of enterocytes [6,20,52].

M cells of Peyer's patches may also be employed for targeted delivery of NPs. It has been shown that fluorescent microparticles can be absorbed rapidly into the mesenteric lymph (within 5 min of administration), albeit in small amounts [53,54]. Coating of particles with certain antibodies may potentially enhance M cell absorption [53,55].

Enhanced paracellular transport: Molecules larger than 1 nm are blocked by the intercellular TJs [6,56], hence, these junctional complexes must be reversibly opened by polymers and calcium chelators to allow transport between adjacent epithelial cells. These polymers include CS (cationic) and PAA (anionic), together with their derivatives, which induce a cascade of reactions that eventually result in TJ disassembly. On the other hand, calcium chelators disrupt TJs via activation of protein kinase C [1,6,57].

Development of Nanoparticle Formulations for Delivering Insulin to Intestinal Epithelium

NP carriers must have a balance of characteristics to allow effective insulin delivery. Their size should be small enough to allow diffusion and uptake, but large enough to carry a favorable amount of insulin. Mucoadhesion prolongs the residence time of NPs at absorption sites, but can hinder diffusion through the GI mucus layer [1]. Various formulations have been tested, and some subsequently modified to find the right composition for efficient oral delivery of insulin. These are summarized in Table 1.

Polymeric nano-carriers

Chitosan (CS): A cationic polysaccharide synthesized by partial deacetylation of chitin (polymer found in crustacean shells and insects) [6,91], consists of glucosamine and N-acetyl-glucosamine, with varying proportions depending on the degree of deacetylation [6,92].

NP carriers composed of CS are more effective to transport insulin than the simple solutions of CS and insulin. CS/insulin NPs caused prolonged hypoglycemia in diabetic rats, unlike the solution form [6,58]. CS is mucoadhesive due to its inherent cationic charges, thus exhibiting electrostatic attraction with the negatively-charged mucus (due to the presence of sialic-acid groups in mucin) [6,93]. Furthermore, CS-based nano-carriers have the ability to cause reversible opening of TJs, increasing paracellular permeation through intestinal epithelium. This phenomenon has been proven in Caco-2 cell monolayers, whereby actin filaments redistribution and transepithelial electrical resistance (TEER) reduction were observed [6,10,59-61]. The reversible disruption of TJs involves CLDN4 degradation and re-synthesis, although it is

likely that other mechanisms exist [6,94]. However, this increase in paracellular permeability is likely to be safe since it did not result in increased absorption of lipopolysaccharide (LPS), which is a common toxin found within the GI tract. This indicates that TJ disruption is not associated with increased permeability to endotoxins [95].

Modifications of CS NP preparations, NP formulations consisting of CS and poly(γ -glutamic acid) (γ PGA) were studied for oral insulin therapy. These CS/ γ PGA NPs were pH-sensitive and increased insulin absorption in the intestine. Results displayed a slow onset, prolonged hypoglycemia in diabetic rats, with a relative bioavailability (compared to SC insulin) of 15%, but increased to 20% if the NPs were in enteric-coated capsules [6,10,60,62,63]. When CS and γ PGA were blended with tripolyphosphate (TPP) and $MgSO_4$, multi-ion-crosslinked NPs were formed. These are more compact, stable over broader pH ranges, and more capable of transporting insulin than simple NPs [59]. Studies suggest that insulin transported in CS/ γ PGA NPs may be used as alternatives to SC basal intermediate-acting insulin [10].

A recent study investigated a further modification of CS/ γ PGA NPs by covalent conjugation of diethylene triamine pentaacetic acid (DTPA) to γ PGA. DTPA acts by chelation of metal cations, including calcium and zinc ions. As a result, chelation of Ca^{2+} causes TJ disruption, enhancing paracellular permeability. Since intestinal metallo-peptidases in the lumen as well as on the brush border require certain metallic ions (e.g. Ca^{2+} and Zn^{2+}) in their structure for enzymatic function, chelation of these cations by DTPA inhibits their proteolytic activity. As a result, CS/ γ PGA-DTPA NPs increased insulin relative oral bioavailability to 20% (in comparison to SC insulin), causing prolonged hypoglycemia in diabetic rats [64].

Chitosan derivatives: The favorable mucoadhesive and TJ opening properties of CS are lost at pH 7.4 due to loss of solubility as a result of deprotonation of amino groups present in CS at neutral/basic pH ranges [6,96]. In order to improve the characteristics of CS, its chemical structure has been altered to form derivatives, including N-trimethylated CS (TMC), thiolated CS and N-(2-hydroxyl) propyl-3-trimethyl ammonium CS (HTCC), which have been tested as potential carriers for oral insulin [6,66,67,69,97,98].

TMC NPs: TMC is synthesized by conjugation of trimethyl groups to the amino groups in CS, which increases its solubility in neutral/basic pH conditions. A TMC and γ PGA NP formulation has been shown to open TJs in Caco-2 cells, improving paracellular insulin transport. Additionally, TMC/ γ PGA NPs allow insulin absorption along the entire intestinal tract, instead of being limited to areas with pH values close to the p^{ka} of CS (~6.5), as exhibited by CS/ γ PGA NPs [6,65].

TMC NPs can be further modified to include a targeting ligand, CSKSSDYQC (CSK), which specifically recognizes goblet cells. Since goblet cells are the second most common intestinal epithelial cell, TMC-CSK NPs are potentially useful carriers for oral insulin delivery. Experimental results involving goblet cell-like HT29-MTX cells show that uptake mechanisms of TMC NPs and TMC-CSK NPs involve active transport and electrostatic interaction. The addition of the CSK ligand further enhanced absorption via clathrin- and caveolae-mediated endocytosis. Thus, TMC-CSK NPs produced a greater hypoglycemic effect with an increased relative bioavailability by 1.5-fold compared to unmodified TMC NPs [66].

Thiolated CS NPs: These contain attached thiol groups and can form covalent bonds with mucin glycoproteins via thiol-disulfide

NP formulation		Experimental model	Observation	Reference
CS	CS NPs	Diabetic rats; Caco-2 cell monolayer	Prolonged hypoglycemia; TJ opening effect	[6,58] [6,10,59-61]
	CS-γPGA NPs	Diabetic rats	Prolonged hypoglycemia; BA 15% (20% when the NPs were enteric-coated)	[6,10,60,62,63]
	CS/γPGA-DTPA NPs	Diabetic rats	Prolonged hypoglycemia; BA 20%	[64]
CS derivatives	TMC/γPGA NPs	Caco-2 cell monolayer	TJ opening effect	[6,65]
	TMC-CSK NPs	Caco-2/HT29-MTX co-culture; Diabetic rats	Enhanced absorption via clathrin- and caveolae-mediated endocytosis; Greater hypoglycemic effect; Increase BA by 1.5-fold (compared to unmodified TMC NPs)	[66]
	TMC-Cys NPs	Caco-2 cell monolayer; Rat intestinal model	Improved absorption	[6,67,68]
	HTCC NPs	In vitro	TJ opening effect	[69]
PLGA	PLGA NPs loaded with insulin-PL complex	Diabetic rats	BA 7.7%	[6,70]
	PLGA-HP55 NPs	Diabetic rats	BA 6.27%	[6,71,72]
	PLGA/RS NPs (in HP55-coated capsules)	Diabetic rats	Prolonged hypoglycemia; Pharmacological availability ~9.2%	[73]
	Antacid-incorporated PLGA NPs	Diabetic rats	Oral bioavailability 6 times higher than native insulin	[74]
	CS-PLGA NPs	Diabetic rats	Greater bioadhesion; PA 10.5%	[75]
Acrylic-based	PAA-Cys NPs	Diabetic rats	Increase in mucoadhesivity; 2.3-fold increase in area under the curve of insulin	[6,76]
	PIBCA NPs	Diabetic rats	Significant increase in absorption; Prolonged hypoglycemia	[77]
	PACA NPs	Diabetic rats	Prolonged hypoglycemia (36 h)	[78]
	P(MAA-g-PEG) and P(AA-g-PEG) NPs	Diabetic rats	Protected insulin at low pH; Prolonged hypoglycemic (>6 h); Up to 50% glucose reduction	[79]
	poly(PEGDMA:MAA) microparticles	Diabetic rats	pH-sensitive; Prolonged hypoglycemia (8-10 h)	[80]
PLA	PLA-F127-PLA NPs	Diabetic mice	Prolonged hypoglycemia (23 h)	[6,81]
PCL	PCL/RS NPs	Diabetic rats	52% blood glucose reduction	[6,82,83]
Poly(allylamine)	Insulin-poly(allylamine) NPs	Caco-2 cell monolayer; In vitro	Reduced pepsin, trypsin and chymotrypsin degradation; Improved transcellular and paracellular transport; TJ opening effect	[6,84-86]

Dextran	VB12-dextran NPs	Diabetic rats	Prolonged hypoglycemia (54 h); 70-75% blood glucose reduction; PA 29.4%	[6,45-47]
	DPPC/cholesterol	Normal & diabetic rats	Greater hypoglycemic effect; Protection against degradation in the GI tract and entered the bloodstream intact	[87]
Liposomes	WGA-, TL- and UEA1-modified liposomes	Normal rats & diabetic mice	Hypoglycemic effect; BA 9.12% (WGA), 7.89% (TL) and 5.37% (UEA1)	[88]
	NaTC liposomes	Normal mice	Greater hypoglycemic effect	[89]
SLNs	Cetyl palmitate-based SLNs	Diabetic rats	Prolonged hypoglycemia (24 h); PA 5.1%	[6,90]
	(WGA-N-glut-PE)-modified SLNs	In vitro; Normal rats	Greater protection against enzymatic degradation; PA 6.08%	[51]

Table 1: Oral insulin nano-carriers and experimental results relating to each.

exchange reactions, resulting in increased mucoadhesion. A thiolated CS preparation, TMC-Cys is formed by linking Cys via amide bonds to primary amino groups on TMC. When used as nano-carriers, TMC-Cys improved absorption of orally administered insulin in Caco-2 cells and rat intestinal models. Moreover, TMC-Cys NPs showed no evidence of toxicity in biocompatibility assessments [6,67,68].

HTCC NPs: A form of quaternized CS, HTCC, was shown to exhibit greater cationic charge density, increased mucoadhesion, as well as TJ opening ability. The disruption of TJs occurred via the binding of HTCC to zona-occludens proteins and redistribution of actin filaments. Moreover, ELISA results indicated that the activity of insulin was preserved in HTCC microparticles, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays proved that the microparticles were non-cytotoxic [69]. Hence, HTCC carriers may also be used to aid oral insulin delivery.

Poly(lactide-co-glycolide) (PLGA): PLGA particles are known to be biocompatible [6,99]. Insulin has been combined with soybean phosphatidylcholine to form insulin-phospholipid complex which can be loaded into PLGA NPs. This oral preparation managed to cause prolonged hypoglycemia (42.6% blood glucose reduction) in diabetic rats for up to 12 h post-administration. Relative bioavailability (compared to SC insulin) was 7.7% [6,70].

The burst effect of insulin release can be alleviated by different preparations of PLGA formulations including a star-branched form of PLGA, β -cyclodextrin-PLGA (β -CD-PLGA), as well as PLGA-hydroxypropyl methylcellulose phthalate (PLGA-HP55). PLGA-HP55 NPs was used to deliver insulin with a relative bioavailability of 6.27% in diabetic rats [6,71,72].

PLGA and Eudragit® RS NPs: Another study reported on the manufacturing of NPs composed of PLGA and Eudragit® RS (RS), a polycationic mucoadhesive acrylic polymer [6,73]. These PLGA/RS NPs containing insulin were filled in hard gelatin capsules coated with HP55, a pH-sensitive polymer. This led to a two-stage delivery mechanism. First, the HP55-coated capsules protected the NPs against low pH and enzymatic damage in the gastric environment. Second, the mucoadhesive PLGA/RS NPs attached to intestinal epithelium and released insulin. RS contains positively-charged ammonio-methyl

groups that can attach to mucus. The NPs may also be able to open TJs to release insulin that can be absorbed paracellularly. The resulting effect is prolonged hypoglycemia in diabetic rats, with a pharmacological bioavailability of insulin around 9.2% [73].

Antacid-incorporated PLGA NPs: Since insulin has been reported to be unstable in PLGA microparticles due to deamidation, antacids (e.g. magnesium hydroxide, magnesium carbonate or zinc carbonate) can be incorporated to form antacid-insulin co-encapsulated PLGA NPs. This method of preparation preserved the structure of insulin, and protected it under simulated gastric and intestinal fluids. The antacid-incorporated NPs exhibited an oral bioavailability 6 times higher compared to native insulin, and caused dose-dependent hypoglycemia in diabetic rats [74].

CS-PLGA NPs: Recently, PLGA NPs have been cationically modified by coating them with CS, since the anionic surface charge of PLGA limits its oral bioavailability. CS-PLGA NPs had greater bioadhesion and improved the relative pharmacological activity of oral insulin (compared to SC) from 7.6% (in PLGA NPs) to 10.5% (in CS-PLGA NPs). MTT assays proved that there was no increased toxicity as well. These improved characteristics by the CS coating might allow CS-PLGA nano-formulations to be successful as oral insulin nano-carriers [75].

Acrylic-based polymers and copolymers: Evidence suggests that poly(acrylic acid) (PAA) and thiomers show mucoadhesive properties [6,100,101]. An experiment combining these two materials to form thiolated PAA-based particles, in the form of PAA-Cys NPs as vehicles for oral insulin, caused hypoglycemia in diabetic rats. There was also 2.3-fold increase in the area under the curve of insulin with PAA-Cys compared to unaltered PAA particles. This suggests that thiolation of PAA increases its mucoadhesive properties [6,76].

Poly(isobutylcyanoacrylate) (PIBCA) nano-dispersions is another nano-carrier tested for oral insulin delivery, which showed statistically significant increase in absorption in PIBCA-encapsulated insulin, compared to that of native insulin [77]. Other forms of poly(alkylcyanoacrylate) (PACA) NPs dispersed in microemulsion was also tested for administering insulin intragastrically to diabetic rats, causing considerable hypoglycemia for up to 36 h [78].

Acrylic-based hydrogels exhibit different levels of efficacy for insulin transport depending on their network structure formed during the polymerization process. A study showed that P(MAA-g-EG) hydrogels had the maximum bioavailability when prepared using the least amount of solvent [102].

Gel nanospheres were manufactured containing crosslinked networks of methacrylic acid (MAA) or acrylic acid (AA), both grafted with poly(ethylene glycol) (PEG). These P(MAA-g-PEG) and P(AA-g-PEG) nanospheres were used to deliver insulin orally in diabetic rats. The entrapped insulin was protected at low pH, and released at neutral pH conditions. Hypoglycemic effects on diabetic rats lasted for at least 6 h. In addition, amongst the gel nanosphere formulations investigated, P(AA-g-PEG) prepared from a molar ratio of 2:1 AA:EG caused the most hypoglycemia (almost 50% glucose reduction) [79].

Another acrylic-based hydrogel used in the oral delivery of insulin is in the form of microparticles based on copolymerization of PEG dimethacrylate (PEGDMA) with MAA. PEG of various molecular weights (400-4000 g/mol) were used in the synthesis of poly(PEGDMA:MAA). These hydrophilic, pH-sensitive poly(PEGDMA:MAA) microparticles held on to insulin at acidic conditions (pH 2.5) and released insulin at neutral/basic environments (pH 7.4). When administered to diabetic rats, prolonged hypoglycemia was observed up to 8-10 h. The copolymers made from the heaviest PEG substrate (molecular weight of 4000 g/mol) exhibited the highest efficiency in insulin transport, since with larger molecular weights, microparticles had a decrease in cross-linking density, greater hydrophilicity, and hence, higher insulin loading capacity [80].

Poly(lactide) (PLA): PLA-b-Pluronic-b-PLA (PLA-F127-PLA) vesicles were tested as insulin delivery vehicles, orally administered to diabetic mice. Hypoglycemia lasted for 23 h, with a maximum glucose reduction at 4.5 h [6,103]. Pluronic block copolymers contain polyethylene oxide blocks which are attracted to the small intestine. They are also amphiphilic, and thus, able to pass through cell membranes [6,81].

Poly(ϵ -caprolactone) (PCL): NPs made up of PCL (polyester) and Eudragit® RS have been successfully tested for oral insulin delivery. Insulin in its regular hexameric form, as well as a monomeric insulin analog (aspart-insulin), was tested using this NP preparation. Insulin activity was preserved and a reduction in hyperglycemia was induced, with maximum effect 8 h post-administration. However, the effect on blood glucose level was greater with the NPs containing aspart-insulin (-52%) than the NPs with regular insulin (-24%), suggesting that aspart-insulin is absorbed more effectively [6,82,83].

Poly(allylamine): These polymers are amphiphilic and can incorporate insulin by forming nano-complexes. These NPs are able to minimize enzymatic degradation of insulin by pepsin, trypsin and chymotrypsin *in vitro*. Additionally, transcellular and paracellular transport of insulin across Caco-2 cell monolayers were improved with the latter being via reversible disruption of TJs [6,84-86].

Dextran: Dextran conjugated to vitamin B12 has been used for targeted delivery of oral insulin. Vitamin B12 complexes with intrinsic factor (IF) in the intestine, and is recognized by IF-specific receptors on enterocytes. Results indicated better transport, in the form of higher pharmacological availability, of NPs made up of dextran-B12 compared to unmodified dextran. Hypoglycemia was detected, lasting for 54 h [6,45-47].

Lipid-based nano-formulations

Liposomes: Liposomes are made up of concentric lipid bilayers, interspersed with aqueous spaces, are able to protect molecules entrapped within them [87,104]. Insulin loaded into liposomes composed of egg phosphatidylcholine/cholesterol and phosphatidylinositol mildly reduced blood glucose levels in rats [87].

Dipalmitoyl-phosphatidylcholine (DPPC)/cholesterol liposomes managed to cause more significant hypoglycemia in normal and diabetic rats. DPPC liposomes also offered some protection against degradation within the GI tract and entered the bloodstream intact [87].

Modified liposome preparations: Studies on modification of liposomes have been carried out which showed improvements in its stability within the GI environment. Sugar chain portions of mucin and PEG were used as surface coatings on liposomes, to form Mucin-Lip and PEG-Lip, both of which were loaded with insulin and orally administered to rats. Surface-modified liposomes, when compared to uncoated liposome preparations, were better able to survive digestion against bile salts in the GI tract and also caused a gradual but more sustained hypoglycemic effect [105].

Lectins such as wheat germ agglutinin (WGA), tomato lectin (TL) and Ulex europaeus agglutinin 1 (UEA1) can be incorporated into liposomes containing insulin, in order to target specific sites on intestinal epithelium. WGA is known to attach to Caco-2 cell membranes and taken up by endocytosis, whereas TL exhibits resistance to enzymatic action in the intestine, as well as having affinity to rat intestinal mucosa. On the other hand, UEA1 has been shown to have specificity to M cells of Peyer's patches in mice. When included in the preparation of insulin-containing liposomes, WGA-, TL- and UEA1-modified liposomes caused hypoglycemia in rats, with relative bioavailabilities of 9.12%, 7.89% and 5.37% respectively (compared to SC insulin). No hypoglycemia was observed with unmodified insulin-containing liposomes [88].

Permeation enhancers (e.g. sodium taurocholate, NaTC) can be incorporated into the insulin-liposome preparation. NaTC is known to disrupt TJs, enhancing paracellular transport. Insulin-NaTC liposomes caused larger glucose reductions in mice compared with insulin liposomes [89].

Solid lipid nanoparticles (SLNs): Cetyl palmitate-based SLNs have been loaded with insulin and orally administered to diabetic rats, with a relative pharmacological bioavailability of 5.1% (compared to SC insulin) and caused significant hypoglycemia over 24 h [6,90]. Another study investigated lectin-modified SLNs as insulin carriers, in which the SLNs were modified with wheat germ agglutinin-N-glutaryl-phosphatidylethanolamine (WGA-N-glut-PE). In comparison with insulin-loaded SLNs, WGA-modified SLNs exhibited greater protection for insulin against enzymatic degradation *in vitro*, as well as increased its relative pharmacological bioavailability (compared to SC administration) from 4.46% (in SLNs) to 6.08% (in WGA-modified SLNs) [51].

Other lipid-based formulations: A delivery vehicle made up of a polyelectrolyte complex of insulin-CS, dispersed in a water-in-oil microemulsion, was tested on diabetic rats. This formulation protected the incorporated insulin from enzymatic degradation in simulated gastric environments. The biological activity of insulin was preserved

and prolonged hypoglycemia was observed in diabetic rats receiving the oral preparation [106].

Other nanoparticle formulations

Multilayered NPs: A complex multilayered NP preparation made of a hydrogel consisting of alginate, dextran sulfate and poloxamer 188, was successfully manufactured. Insulin was incorporated into the hydrogel, stabilized by CS and coated with albumin [107-109]. Poloxamers, also known as pluronic block copolymers, are made up of hydrophilic poly(ethylene oxide) and hydrophobic poly(propylene oxide) blocks, and have the ability to increase drug solubility and transport in the intestine [1,110,111].

In vitro studies on this complex hydrogel formulation showed that the preparation procedure maintained the bioactivity of insulin. Moreover, the characteristics of the insulin-NP complex could be adjusted depending on minute variations of reactant concentrations. Reducing the concentrations of calcium ions while increasing poloxamer and albumin concentrations resulted in smaller NPs. Reduction in albumin content, however, improved insulin entrapment and retention when exposed to simulated gastric conditions. The authors concluded that the optimum proportions of the components were 0.20% calcium chloride, 0.06% alginate, 0.04% dextran sulphate, 0.04% poloxamer 188, 0.006% insulin, 0.07% CS and 0.5% albumin [107].

Further studies on this multilayered NP formulation showed that insulin was protected against enzymatic digestion and its absorption enhanced. Insulin transport was increased by the NP preparation (compared to free insulin) as much as 2.1-fold through Caco-2 cell monolayer, 3.7-fold through Caco-2/HT29 co-culture (mucus secreting), and 3.9-fold through rat intestinal models. This resulted in sustained (>24 h) hypoglycemia (up to 40% of basal levels) in diabetic rats, as well as 13% bioavailability [108,109].

Conclusion

There are great benefits of successful oral insulin delivery, in terms of its ease and simplicity, improved patient compliance, ultimately leading to better patient outcome. Thus, tremendous efforts have been made to develop the best oral insulin delivery device. Nanotechnology has provided a promising platform for engineering such insulin carriers.

Nevertheless, it is important to note that experimental results involving the use of animal models need to be interpreted with care, due to the differences between the anatomy and physiology of laboratory animals and humans. For example, compared to humans, rats and mice produce less mucin. Rodents also have a less acidic gastric fluid. Furthermore, there may be other differences that would significantly affect the transport of the many NP carriers tested so far [20,112-115].

To date, there have been no approved oral insulin nano-formulations. This is probably due to the difficult requirements that need to be met by the drug preparations. In order to be successful, orally administered insulin nano-carriers must be able to increase insulin bioavailability to reach therapeutic levels, with minimal variation amongst different individuals, as well as having a financially feasible manufacturing process for mass production [1]. Another potential problem is that high insulin doses may induce mitogenic changes in the GI epithelium since insulin is a growth factor [6,7]. In other words, an efficient delivery system should require lower insulin doses to achieve therapeutic hypoglycemic effects [6].

Possible future directions in oral insulin research include alterations to insulin in order to achieve greater stability [6,8,12], as well as combination therapy where insulin substitutes (e.g. glucagon-like peptide 1 and exenatide) may be co-administered with insulin for more effective glycemic control but with fewer side effects [6,116]. Since there have been many new investigations and discoveries of potential NPs to administer insulin orally, and these formulations are continuously being improved, the future of oral insulin looks promising.

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