



Recent Advances in Insulin Therapy for Diabetes

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Abstract

Diabetes mellitus is a chronic disease affecting a great number of the world's population. Insulin therapy for diabetes is most commonly delivered via subcutaneous injections, up to four times a day. Long-term insulin therapy, compounded by the invasive nature of its administration, has caused problems with patient compliance, ultimately influencing patient outcomes. Extensive research has been carried out to date, to explore possible improvements to insulin therapy for diabetic patients, with some new products already available and FDA-approved. This review aims to highlight the new discoveries in novel insulin formulations, such as novel injectable insulins, artificial pancreas systems, oral insulins, transmucosal insulins including buccal insulins, pulmonary/inhaled insulins, nasal insulins, ocular insulins, and rectal insulins, as well as transdermal insulins, from preliminary research data to market available insulin products, and finally discuss potential future directions of insulin therapy.

Keywords

Diabetes, Insulin, Insulin delivery, Injectable insulin, Oral insulin, Transmucosal insulin, Buccal insulin, Pulmonary insulin, Inhaled insulin, Nasal insulin, Ocular insulin, Rectal insulin, Transdermal insulin

Introduction

Diabetes mellitus (DM) is a chronic disease that is caused by defective pancreatic insulin production – type 1 DM (T1DM, previously known as insulin-dependent or juvenile-onset DM), or insulin resistance – type 2 DM (T2DM, previously known as non-insulin-dependent or adult-onset DM). The disease results in hyperglycaemia which can lead to multi-organ damage in the long run, e.g. vasculopathy, neuropathy, etc. DM causes significant rates of morbidity and mortality to the current population [1].

Insulin is used to control the level of blood glucose in patients with DM. It is an essential therapy for patients suffering from T1DM, and T2DM (especially in late-stage disease). Insulin was discovered in 1921 by Frederick Banting and Charles Best, went through its first clinical use in 1922 [2-4], and helped revolutionise the treatment of T1DM which was fatal at that time. Initially, insulin was isolated from bovine and porcine pancreata, until the 1980s. Later on, recombinant DNA techniques allowed the manufacture of human insulin. Modifications of the amino acid sequence of the insulin molecule by rDNA and protein engineering methods have recently

allowed the production of monomeric insulin analogues (e.g. lispro, aspart) which have a more rapid absorption profile [3].

Since DM results in a defect in insulin function, the ideal treatment is to allow diabetics to regain normal insulin function. However, current research and technology has not been able to achieve this. The current insulin treatment involves exogenous administration, with the aim of achieving effective glycaemic control (i.e. prevention of hyper- and hypoglycaemia) and avoidance of the complications of DM [5]. Current modes of delivering insulin include intravenous (IV) infusion and subcutaneous (SC) injections. SC insulin preparations, which are more commonly used, include rapid-, intermediate- and long-acting insulin, which are used in different combinations (1 to 4 times or more daily) [3]. Table 1 lists the different insulin preparations together with their durations of action.

Disadvantages of current approved insulin regimens relate to the injectable mode of its administration. Daily injections have led to non-compliance, which has obvious effects on patient outcomes. In addition, parenteral administration bypasses the liver, unlike physiologically-secreted insulin. Hepatic first-pass metabolism, which decreases the amount of circulating insulin by approximately 50% in normal individuals, prevents peripheral hyperinsulinaemia and its associated complications [5-11].

Advances in Insulin Therapy

The recent advances in novel insulin therapy for diabetes include newer injectable formulations, artificial pancreas systems, oral, transmucosal, and transdermal dosage forms. These are covered in more detail under the following subheadings and are summarized in Table 2.

Injectable insulin

Newly discovered parenteral insulin formulations include a different insulin glargine concentration of 300 U/ml, an ultra-long acting insulin degludec, as well as ultra-short acting insulin Linjeta™. Both formulations show favourable characteristics that may replace or supplement currently available insulin injections.

Insulin glargine 300 U/ml (IGlar-300): 2 randomized controlled trials compared the efficacy of 2 different doses of insulin glargine, 300U/ml (IGlar-300) and 100U/ml (IGlar-100), on patients with T2DM. One study involved T2DM patients using basal and mealtime insulin (EDITION 1 study) [12], whereas another

Citation: Ahmad A, Othman I, Md Zain AZ, Chowdhury EH (2014) Recent Advances in Insulin Therapy for Diabetes. *Int J Diabetes Clin Res* 1:006

Received: October 01, 2014; **Accepted:** October 23, 2014; **Published:** October 27, 2014

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Table 1: Durations of action of different insulin preparations.

Insulin preparation		Onset (h)	Peak (h)	Duration (h)
Rapid-acting	Insulin analogues: lispro, aspart, glulisine	< 0.5	0.5 – 2.5	3 – 4.5
Short-acting	Regular, soluble insulin	0.5 – 1	1 – 4	4 – 8
Intermediate-acting	Isophane (NPH), lente	1 – 3	3 – 8	7 – 14
Long-acting	Bovine ultralente	2 – 4	6 – 12	12 – 30
	Insulin analogues: glargine, detemir	1 – 2	None	18 – 24

These are classified into rapid-, short-, intermediate- and long-acting insulin [3].

Table 2: Newer insulin formulations and details of their respective experimental results.

Insulin formulation		Experimental model	Results	Reference
Injectable	Insulin glargine 300 U/ml	Human studies (T2DM), randomized controlled trial	Less risk of nocturnal hypoglycaemia, with no increase in risk of daytime hypoglycaemia [vs. IGlar-100]. Similar extent of Hb _{A1C} control [IGlar-300 vs. IGlar-100].	[12,13]
	Insulin degludec	Human studies (T1DM, T2DM)	Better glycaemic control [vs. IGlar&IDet]. Less hypoglycaemic episodes, including nocturnal hypoglycaemia [vs. IGlar&IDet]. Flexibility in timing of doses. Non-inferior to IGlar in achieving Hb _{A1C} of 7%. May increase MACE risk.	[5,14-31]
	Linjeta™ (VIAject™)	Human studies (healthy, T1DM, T2DM)	Faster onset [vs. human soluble insulin & LIS]. Less within-subject variability, less postprandial glycaemic excursions & oxidative stress, improved endothelial function [vs. RHI & LIS].	[5,32-36]
Artificial pancreas	Delivery system involving CGM, CSII, and an algorithm (MPC) <i>Using short- or ultrashort-acting insulin ± absorption enhancers</i>	Human studies (T1DM)	A newer 'smart' MPC algorithm allows more accurate glycaemic control – faster response & less extreme glucose fluctuations [vs. PID algorithm]. Outpatient studies indicate CLC systems are safe & efficient for clinical practice.	[5,37-39]
Oral	Nanoparticle-based: <i>CS & CS-derivatives, PLGA, acrylic-based, PLA, PCL, poly(allylamine), dextran, lipid-based (e.g. liposomes & SLNs), multi-layered NP</i>	<i>In vitro, in vivo</i> (normal & diabetic mice/rats)	Improved absorption & bioavailability. Greater & prolonged hypoglycaemic effect.	[10]
	Nanolayer encapsulation of insulin-CS complexes	<i>In vitro, in vivo</i> (STZ-induced diabetic mice)	Sustained-release of insulin. Improved insulin loading capacity [>90%], insulin stability, 74% less solubility at low pH [vs. non-encapsulated insulin]. Decreased fasting glucose levels by up to 50% [sustained & dose-dependent]. Decreased postprandial hyperglycaemia, without risk of hypoglycaemia.	[40]
	Nanoparticulated insulin in multiple emulsion form	<i>In vitro</i>	Good release characteristics. Protected insulin at different pH levels.	[41]
	Insulin-loaded alginate/CS blend gel beads cross-linked by glutaraldehyde	<i>In vitro</i>	Greater stability in SGF & SIF. Reduced cumulative insulin release in SGF. Improved performance of gel beads in SIF.	[42]
	IN-105	Animal studies, human studies (Phase 2 trials)	Improved half-life & absorption. Lower immunogenicity & mitogenic effect. Similar pharmacological effect. Wide therapeutic window. Dose-dependent postprandial glycaemic effects.	[5,43,44]
Buccal	Oral-lyn™	Clinical trials (market available)	Dose-dependent absorption profile. Faster onset & shorter duration of action [vs. SC insulin].	[5,45]
	Insulin-coated NP loaded in ERL films	<i>In vitro</i> (EpiOral human buccal mucosa model)	Enhanced permeation through EpiOral model.	[46]
	Insulin-loaded NP embedded into CS films	<i>In vitro</i> (EpiOral human buccal mucosa model)	Enhanced permeation through EpiOral model by 1.8-fold [vs. pure insulin].	[47]
	Insulin-bearing pelleted bioadhesive polymeric NP	<i>In vivo</i> (diabetic rats)	Significant hypoglycaemia after 7h. No risk of hypoglycaemia.	[48,49]
	Insulin in transferosome vesicles	<i>In vivo</i> (rabbits)	Improved insulin delivery [vs. conventional vesicles]. Relative pharmacological activity of 15.59% [vs. SC insulin].	[48,50]
	Insulin + soybean lecithin + propanediol	<i>In vivo</i> (diabetic rabbits, diabetic rats)	Significant hypoglycaemic effect, lasting 4-5h.	[5,48,51]
	Insulin + PF-127 (containing unsaturated fatty acids)	<i>In vivo</i> (normal rats)	Improved insulin release with fatty acids. Continuous hypoglycaemia. Oleic acid showed best pharmacological availability of 15.9%.	[48,52]
Insulin + lysalbinic acid	<i>In vitro</i> (hamster cheek pouch model)	Increased permeability to insulin.	[48,53,54]	

Pulmonary (inhaled)	Afrezza®	Clinical trials (market available)	Acceptable Hb _{A1C} reduction in T1DM when used pre-meals with another basal insulin, but less Hb _{A1C} reduction [vs. insulin aspart]. Greater Hb _{A1C} reduction in T2DM when used with other OHA [vs. placebo]. May cause bronchospasm in asthma & COPD.	[55]
	Insulin microcrystals	<i>In vivo</i> (STZ-induced diabetic rats)	Prolonged hypoglycaemic effects over 7h. Addition of zinc increases hypoglycaemic effect [17% minimum reductions in blood glucose].	[48,56,57]
	Insulin + HA dry powder + (Zn ²⁺ or HPC)	<i>In vivo</i> (beagle dogs)	Addition of Zn ²⁺ & HPC improved mean residence time by >9-fold & >7-fold, respectively [vs. spray dried pure insulin].	[48,58]
	Insulin + DPPC	<i>In vivo</i> (rats)	Greater hypoglycaemic effect [vs. insulin + liposome].	[48,59]
	Insulin + (bacitracin, Span 85, or citric acid)	<i>In vivo</i> (rats)	Bacitracin & Span 85 improved insulin solution bioavailability to ~100% [not effective in dry powder forms]. Citric acid increased the hypoglycaemic effect, with bioavailabilities of 42-53% for dry powders. No acute toxicity to lung cells by citric acid.	[48,60]
	Insulin + (TDM or DMβCD)	<i>In vivo</i> (rats)	Relative bioavailabilities: [TDM] 0.34-0.84% [DMβCD] 0.19-0.48%. Both have reversible effects on respiratory epithelium [normalises 120min post-exposure].	[48,61]
	Insulin + H-MAP	<i>In vivo</i> (rats)	Dose-dependent effect [maximum at 16mg/kg H-MAP & 1.3 U/kg insulin]. At maximum doses: relative bioavailability increased by >2.5-fold, maximum insulin concentration by 2-fold, blood glucose reduction by 2-fold [vs. same dose of insulin alone].	[62]
	Insulin/liposome	<i>In vivo</i> (alloxan-induced diabetic rats)	Homogeneously distributed throughout lung. Increased drug retention times. Hypoglycaemic effect.	[48,63]
	Insulin-CAP-PEG particle suspensions	<i>In vivo</i> (rats)	Increased half-life & residence times [vs. insulin solution]. Spray instillation was more efficient than intratracheal instillation.	[48,64]
	Insulin/PLGA nanospheres	<i>In vivo</i> (guinea pigs)	Substantial prolonged hypoglycaemic response over 48h [3.9 IU/kg insulin] [vs. 6h by aqueous insulin].	[48,65]
	Insulin/PBCA NP	<i>In vivo</i> (healthy rats)	Improved bioavailability. Significant hypoglycaemic responses. Minimum blood glucose concentrations of 46.9/ 30.4/13.6% of initial levels for 5/10/20 IU/kg insulin in PBCA NP.	[48,66]
Nasal	Insulin + alkylglycosides <i>dodecylmaltoside, tridecylmaltoside, tetradecylmaltoside, ordodecylsucrose</i>	<i>In vivo</i> (hyperglycaemic rats)	Improved insulin absorption. Rapid hypoglycaemic effects, maximum between 60-120min post-administration. Tetradecylmaltoside increases insulin absorption even when administered 15min before insulin.	[48,67,68]
	Insulin + 0.5% sucrose cocoate	<i>In vivo</i> (rats)	Improved insulin absorption, with resulting hypoglycaemic effects.	[69]
	Insulin + carbopol-based gel	<i>In vivo</i> (rabbits)	Significant hypoglycaemic effect. Relative bioavailability of 20.6% [vs. IV insulin].	[48,70]
	Insulin + 2% CS gel + EDTA	<i>In vivo</i> (diabetic rabbits)	Improved insulin absorption. Hypoglycaemic effect up to 46% of that caused by IV insulin.	[48,71]
	Insulin/CS microspheres <i>400mg CS + 70mg ascorbylpalmitate (as cross-linker)</i>	<i>In vivo</i> (diabetic rats)	Absolute bioavailability of 44%. 67% decrease in blood glucose [vs. IV insulin].	[48,72]
	Insulin + AGMS	<i>In vivo</i> (rats)	Insulin released slower from AGMS [cumulative release of 18.4% within 30min, 56.9% within 8h], compared to GMS [cumulative release of 32.4% within 30min, 75.1% within 8h]. AGMS increased nasal insulin absorption significantly when given in dry powder form.	[48,73]
	Insulin/CS-TBA microparticles + reduced glutathione (permeation mediator)	<i>In vivo</i> (rats)	Controlled-release of insulin over 6h. Absolute bioavailability of 7.24% [higher than using unmodified CS].	[48,74,75]
	Insulin/PEGylated TMC NC	<i>In vivo</i> (rats)	Reduced blood glucose levels by 34-47%. Less toxic on nasal epithelium [vs. non-PEGylated].	[76]
	Insulin/NC <i>Amine-modified poly(vinyl alcohol)-graft-poly(L-lactide)</i>	<i>In vivo</i> (healthy & STZ-induced diabetic rats)	50% decrease in blood glucose within 50-80min [fasted healthy rats]. 30% decrease in blood glucose within 75-95min [STZ-induced diabetic rats]. No histological evidence of nasal mucosal damage 4h post-administration.	[77]
	Insulin/p(LAMA-r-AAPBA) NP	<i>In vitro & in vivo</i> (diabetic rats)	Modification of glycopolymers affects insulin release. Good cytocompatibility. NP internalisation via clathrin- & lipid raft/caveolae-mediated endocytosis. Significant hypoglycaemic effect.	[78]

Ocular	Insulin solution + (POELE, NaGC, NaTC, or NaDC)	<i>In vivo</i> (albino rabbits)	Bioavailability ranging from 3.6-12.6% [vs. 0.7-1.3% without absorption enhancers].	[48,79]
	Insulin solution + alkylglycosides <i>tetradecylmaltoside, tridecylmaltoside, dodecylmaltoside, or dodecylsucrose</i>	<i>In vivo</i> (rats)	Improved insulin absorption, at $\geq 0.125\%$ conc. [vs. NaGC requiring $\geq 0.5\%$ conc.].	[48,80]
	Insulin + (fusidic acid or GC)	<i>In vivo</i> (rabbits)	Improved insulin absorption, especially at higher pH [pH 8 vs. pH 3.5].	[81]
	Insulin + absorption enhancer <i>saponin, POELE, Brij-78, fusidic acid, dodecylmaltoside, or tetradecylmaltoside</i>	<i>In vivo</i> (healthy cats, healthy dogs)	Improved insulin absorption, with resulting hypoglycaemic effects [vs. native insulin].	[48,82,83]
	Insulin-loaded liposomes	<i>In vivo</i> (healthy rabbits)	Significant hypoglycaemic effects 90-120min post-administration.	[84]
	Gelfoam® ocular insert containing insulin	<i>In vitro & in vivo</i>	Significant prolonged insulin delivery. Improvement in insulin activity & prolonged duration of action with the addition of Brij-78. No risk of hypoglycaemia. Return to near normoglycaemia 60min after device removal. 5% acetic acid or 1% HCl improved insulin absorption without enhancers.	[48,85-89]
Rectal	Suppositories containing 50U insulin + DCA + NaTC + polycarbophil	<i>In vivo</i> (alloxan-induced hyperglycemic rabbits)	Relative hypoglycaemia of 38% [DCA], 34.9% [NaTC], 44.4% [DCA+NaTC], 56% [DCA+NaTC+polycarbophil] [vs. 40U SC insulin]. ~50% of the efficacy of SC insulin.	[48,90]
	Suppositories (Witepsol W35 base) containing 5U/kg insulin + (50mg sodium salicylate or 1% POELE)	<i>In vivo</i> (diabetic beagle dogs)	Improved insulin absorption. Relative hypoglycaemia of 49-55% [vs. 4U/kg SC insulin].	[48,91]
	Suppositories (Witepsol W35 base) containing 5U/kg insulin + (NaDC+NaC, NaTDC, or NaTC)	<i>In vivo</i> (diabetic beagle dogs)	Relative hypoglycaemia of ~50% [vs. SC insulin]. Adjusting insulin dose allows desirable hypoglycaemic effect according to degree of hyperglycaemia.	[48,92]
	Insulin-CS gel + 5% DM β CD	<i>In vivo</i> (rabbits)	Prolonged insulin release. Maximum hypoglycaemic effect in insulin-CS gel + 5% DM β CD. Improves transvaginal insulin delivery as well.	[93]
	Suppositories containing 100U insulin + 200mg sodium salicylate	Human studies (healthy, T1DM)	Hypoglycaemic effects by 15min, lasting up to 90min post-administration	[94]
	Suppositories containing 200U insulin + 100mg NaC	Human studies (healthy, T1DM)	Maximum of 47.7% reduction in blood glucose levels at 90min post-administration [vs. 50.6% with 20U SC insulin]. Prevented significant postprandial rise in blood glucose in T1DM patients.	[95]
	Thermo-reversible insulin liquid suppository <i>100 IU/g insulin, 15% poloxamer P407, 20% poloxamer P188, 0.2% polycarbophil, and 10% sodium salicylate</i>	<i>In vivo</i> (STZ-induced diabetic rats)	Improved insulin bioavailability Easy to deliver, painless, safe, remained at site of administration.	[48, 96]
Mucoadhesive glycerol-gelatin suppository containing insulin + 7% snail mucin	<i>In vivo</i> (rats)	Sustained-release of insulin. 66% reduction of blood glucose levels within 2h post-administration.	[48, 97]	
Transdermal	Insulin-loaded microemulsions <i>Oleic acid as oil phase, Tween 80 as surfactant, isopropyl alcohol as co-surfactant</i>	<i>In vitro</i> (goat skin)	Formulation of 10% oleic acid, 38% aqueous phase, 50% surfactant phase with 2% DMSO as permeation enhancer showed maximum permeation flux through goat skin	[98]
	Transfersomal gel containing insulin	<i>In vitro</i> (porcine ear skin), <i>in vivo</i> (diabetic rats)	Skin permeation followed zero-order kinetics. Prolonged hypoglycaemic effect >24h].	[99]
	Amidated pectin hydrogel matrix containing insulin	<i>In vivo</i> (diabetic rats)	Delivery of physiologically relevant amounts of insulin, with pharmacological activity.	[100]
	Insulin emulgel <i>carbomer or HPMC (gelling agent) + polysorbate 80 (emulsifier) + emu oil (absorption enhancer)</i>	<i>In vitro & in vivo</i> (albino rabbits)	Hypoglycaemic effect 250 to 185mg/dl at 120min. Adding iontophoresis increased hypoglycaemic effect [250 to 125mg/dl at 120min].	[101]

Abbreviations: T2DM: Type 2 Diabetes Mellitus; IGlar: Insulin Glargine; IGlar-300: Insulin Glargine 300 U/ml; IGlar-100: Insulin Glargine 100 U/ml; T1DM: Type 1 Diabetes Mellitus; IDet: Insulin Detemir; Hb_{A1c}: glycated haemoglobin; MACE: Major Adverse Cardiovascular Events; LIS: Insulin Lispro; RHI: Regular Human Insulin; CGM: Continuous Glucose Monitoring; CSII: Continuous Subcutaneous Insulin Infusion; MPC: Model-Predictive Control; PID: Proportional-Integral-Derivative; CLC: Closed-Loop Control; NP: Nanoparticles; CS: Chitosan; PLGA: Poly(lactide-co-glycolide); PLA: Poly(lactide); PCL: Poly(ϵ -caprolactone); SLN: Solid-Lipid Nanoparticles; STZ: Streptozotocin; SGF: Simulated Gastric Fluid; SIF: Simulated Intestinal Fluid; SC: Subcutaneous; ERL: Trimethylammonioethyl Methacrylate Chloride, Eudragit® RLPO; OHA: Oral Hypoglycaemic Agents; COPD: Chronic Obstructive Pulmonary Disease; PF-127: Pluronic F-127; EDTA: Ethylenediaminetetraacetic Acid; AGMS: Aminated Gelatin Microspheres; GMS: Gelatin Microspheres; CS-TBA: Chitosan-4-Thiobutylamidine; PEG: Poly(ethylene) Glycol; TMC: Trimethyl Chitosan; NC: Nanocomplexes; p(LAMA-r-AAPBA): Poly(2-lactobionamidoethyl methacrylate-random-3-acrylamidophenylboronic acid); HA: Hyaluronic Acid; Zn²⁺: Zinc Ions; HPC: Hydroxypropyl Cellulose; DPPC: 1,2-Dipalmitoyl Phosphatidylcholine; TDM: Tetradecyl- β -maltoside; DM β CD: Dimethyl- β -cyclodextrin; H-MAP: Hydroxy-Methyl-Amino-Propionic Acid; PLGA: Poly(lactide-co-glycolide); PBCA: Polybutylcyanoacrylate; POELE: Polyoxyethylene-9-lauryl Ether; NaGC: Sodium Glycocholate; NaTC: Sodium Taurocholate; NaDC: Sodium Deoxycholate; GC: Glycocholate; Brij-78: Polyoxyethylene-20-stearyl ether; HCl: Hydrochloric Acid; DCA: Deoxycholic Acid; NaTC: Sodium Taurocholate; NaDC: Sodium Deoxycholate; NaC: Sodium Cholate; NaTDC: Sodium Taurodeoxycholate; DMSO: Dimethyl Sulfoxide; CAP: Calcium Phosphate; PEG: Polyethylene Glycol; HPMC: Hydroxypropyl Methylcellulose.

studied the outcomes on T2DM patients using basal insulin and oral antihyperglycaemic drugs (EDITION 2 study) [13]. Both studies showed that IGLar-300 is as effective as IGLar-100 in terms of Hb_{A1C} reduction, but the former is associated with a lower risk of nocturnal hypoglycaemia. The EDITION 1 study found a 10% absolute and 21% relative risk reduction of nocturnal hypoglycaemia, with no increase in daytime hypoglycaemia [12]. Similarly, the EDITION 2 study demonstrated a 23% risk reduction in nocturnal hypoglycaemia ($P=0.038$), in addition to decreased risk of hypoglycaemia at any time (24 hours) [13].

Insulin degludec (IDeg): A new formulation of modified human insulin with ultra-long action, with a half-life exceeding 24 hours [5,21,29]. This novel preparation has been compared with the current basal insulin regimen, insulin glargine (IGlar). Studies show that IDeg, when compared to IGLar, resulted in similar, or even better glycaemic control, with less hypoglycaemic episodes and lower risk of nocturnal hypoglycaemia [5,14-20]. Phase 3 clinical trials on T1DM and T2DM patients indicated that IDeg was similar in efficacy to IGLar in reaching a target Hb_{A1C} of 7%, whilst reducing the risk of nocturnal hypoglycaemia [30]. In addition, IDeg resulted in a greater improvement in overall health status over 2 years [22], with similar Hb_{A1C} control [17], and required lower doses for glycaemic control [19], when compared to IGLar. Although IDeg may require less frequent injections (three times a week) compared to IGLar (once daily) in order to maintain effective basal insulin levels [5,23], more recent studies in T2DM patients do not recommend this thrice weekly IDeg regimen, as it may result in higher risk of hypoglycaemia [24]. However, the time taken to recover from hypoglycaemic episodes back to normal glucose levels was similar in both IDeg and IGLar [25]. Compared to insulin detemir (IDet), IDeg also fared better in terms of glycaemic control, less nocturnal hypoglycaemia and less frequent injections, when used in a basal bolus regimen with insulin as part [5,26,31]. Due to the slow release and achieving a steady-state profile in once daily dosing, IDeg allows for flexibility in timing of doses, without affecting efficacy and safety. This is particularly important to patients who are elderly, with learning difficulties, or those depending on healthcare professionals for insulin injections [29,30]. However, IDeg has not been approved by the Food and Drug Association (FDA) due to facts indicating an increase in the risk for major adverse cardiovascular events (MACE) associated with IDeg [5,27,28].

Linjeta™ (previously VIAject™): This form of recombinant human insulin has an ultra-short action, with faster onset compared to human soluble insulin and insulin lispro (LIS) [5,32,33], and showed less within-subject variability compared to regular human insulin (RHI) [5,34]. When compared to RHI and LIS, Linjeta™ exhibited less postprandial glycaemic excursions and oxidative stress, as well as improved endothelial function [35,36].

Artificial pancreas

Also known as an integrated closed-loop control (CLC), this system utilises real-time feedback from continuous glucose monitoring (CGM), to continuously adjust insulin administration via an insulin pump (continuous subcutaneous insulin infusion, CSII) depending on blood glucose levels [102]. The system consists of a continuous glucose monitor, linked wirelessly to an insulin infusion pump, which delivers insulin in accordance to an algorithm, automatically. This method mimics physiological pancreatic β -cell function to a certain extent, and reduces the risk of hypoglycaemia [5]. It is minimally invasive as the electrode is implanted in the subcutaneous tissue and measures glucose electrochemically [103].

However, this system has limitations due to delays in both insulin action and blood glucose sensing. First, the sensors measure the glucose in the interstitial fluid rather than blood (a delay of 5-15 minutes), which can lead to errors in accuracy? Second, insulin analogues available today have a lag period in their absorption into the bloodstream and, thus, in their onset of action. These may result in insulin action to be delayed by approximately 20 and 100 minutes in peripheral tissues and the liver, respectively. The algorithm used by

the system can help circumvent this delay to some extent. Previous proportional-integral-derivative (PID) control algorithms merely respond to glucose levels, and faced the previously mentioned lag periods. The newer model-predictive control (MPC) algorithm models the patient's metabolic system and reduces this problem by planning the insulin delivery several moves ahead. This 'smart' system suffers less lag periods and extreme blood glucose fluctuations [5,37,38].

There are numerous advancements in this artificial pancreas system to date. The development of rapid-acting insulins such as Linjeta™, and absorption enhancers may help in improving the response time of CLC systems [5]. One such enhancer is a recombinant insulin hyaluronidase (rHuPH20), which improved glucose control after meals and reduced hypoglycaemia in patients with T1DM and T2DM [5,104,105]. Additionally, it has been shown that the control system for insulin delivery can be programmed into a regular smart phone [106,107]. This may make this system less costly, more accessible and more easily upgradable. Studies in outpatient settings with artificial pancreas systems found that the current settings are safe and efficient for day-to-day use. With advancements in technology, even better outcomes can be expected in the future [39].

Oral insulin

The ultimate goal of exogenous insulin therapy is via oral administration, due to its numerous advantages. Firstly, oral delivery of insulin enters the portal circulation and reaches the liver before entering the systemic circulation. This resembles the pathway of physiologically secreted insulin, resulting in inhibition of glucose production by the liver and avoidance of peripheral hyperinsulinaemic effects [5,6,8-11]. Secondly, the inconvenience of invasive (injectable) forms of insulin has led to problems in patient compliance, ultimately affecting patient outcomes [5,7,10,108].

Nevertheless, this ideal insulin formulation has so far been elusive due to the difficulties faced in designing such a delivery system. Orally administered drugs need to survive harsh gastric conditions in which they are exposed to acidic and enzymatic degradation. Subsequently, intestinal conditions further expose the drugs to a myriad of digestive enzymes [5,6,10,109-112]. The drugs that survive these conditions then need to penetrate the mucus layer overlying the absorptive surfaces of the gastrointestinal tract (GIT) and pass through the epithelial cells to enter the blood stream. Insulin, being a peptide molecule, is easily degraded by the conditions in the GIT, which significantly lowers the bioavailability of orally administered insulin [5,6,10].

One method of circumventing this difficulty would be to design an insulin delivery system that would protect it from damage throughout its GIT transit and release the insulin at the absorption sites. Nanotechnology has shown promising results in the design of potential oral insulin nanocomposites. These delivery systems can be designed to protect the insulin molecules from degradation, modify the release profile of insulin from the nanoparticles (NP), and/or target specific absorption sites, ultimately increasing the bioavailability of orally delivered insulin [10].

We have published a review article on current nanodevices for targeted oral insulin therapy in the Journal of Nanomedicine and Nanotechnology [10]. These nanoformulations include those based on chitosan (CS) and CS-derivatives, poly(lactide-co-glycolide) (PLGA), acrylic-based NP, poly(lactide) (PLA), poly(ϵ -caprolactone) (PCL), poly(allylamine), dextran, lipid-based formulations such as liposomes and solid-lipid nanoparticles (SLN), as well as multi-layered NP. *In vitro* and *in vivo* studies on normal and diabetic mice/rats found evidence to indicate that these NP formulations improved insulin absorption and bioavailability via the oral route, resulting in a greater hypoglycaemic effect. Some NP preparations caused a prolonged hypoglycaemic effect as well [10]. We have also studied another inorganic NP known as carbonate-apatite (CO₃Ap), which has the potential to be used as a delivery device to deliver insulin via the oral route. Preliminary data suggests that the insulin-CO₃Ap

composites can be made more stable by incorporation of strontium ions [113].

More complicated nanoformulations have also been experimented upon. For example, nanolayer encapsulation of insulin-CS complexes has been studied for the sustained release of insulin after oral administration. The insulin-CS microparticulate cores were coated with CS/heparin multilayer coatings, with poly(ethylene) glycol (PEG) present in the precipitating and coating solutions. This preparation improved insulin loading capacity (>90%), and increased insulin stability (74% decrease in solubility at acidic pH *in vitro*), relative to non-encapsulated insulin. *In vivo* studies on streptozotocin (STZ)-induced diabetic mice found that this delivery system caused a drop in fasting glucose levels by up to 50%, with a sustained and dose-dependent behaviour. The formulation also decreased the surge in blood glucose levels post-meals, without risk of hypoglycaemia [40]. A separate nanoparticulated insulin in multiple emulsion form also showed promise for oral delivery. Insulin-polymeric NP made using layer-by-layer adsorption method, which is then incorporated into an emulsion, forming a nanoparticulated multiple emulsion, consisting of insulin nanoaggregates between 300 to 400nm in size. These nano-insulin preparation exhibited good release characteristics and offered protection to insulin at different pH levels [41]. Insulin-loaded alginate/CS blend gel beads cross-linked by glutaraldehyde showed favourable characteristics in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). These beads were prepared by dripping a homogeneous solution of alginate and CS into 2% calcium chloride solution, forming calcium cross-linked beads, which in turn, were immersed in 2% glutaraldehyde solution. These dual cross-linked alginate/CS beads exhibited greater stability in SGF and SIF. Due to the cross-linking, the presence of CS was found to reduce the cumulative insulin release in SGF and improved the performance of the alginate/CS beads SIF [42].

These previously-mentioned studies were limited to *in vitro* and *in vivo* experiments. However, a new insulin analogue known as IN-105 is currently being studied in phase 2 trials, by the pharmaceutical company Biocon, in association with Bristol-Myers Squibb [5,114]. IN-105, a conjugated recombinant insulin [115], lasted longer in the GIT, had better absorption, was less immunogenic and less mitogenic compared to insulin, but exhibited similar pharmacological activity. IN-105 also did not show significant genotoxic, mutagenic or teratogenic potential [5], and may have a wide therapeutic window as study subjects showed no clinical hypoglycaemic effects [43]. It also showed a dose-dependent effect on 2-hour postprandial glucose levels [5,43,44].

Transmucosal insulin

Transmucosa insulin delivery include buccal, pulmonary (inhaled), nasal, ocular, as well as rectal routes. These routes are common in that they involve insulin absorption/transport across mucosal surfaces into the bloodstream.

Buccal insulin: Buccal delivery of insulin involves aerosol delivery of the drug into the oral cavity, after which absorption occurs through the inner surfaces of the cheeks and the back of the mouth. This distinguishes it from oral delivery, which is absorbed further along the GIT, as well as pulmonary delivery, which is absorbed in the lungs [5]. The buccal mucosa is relatively immobile, highly vascularised, and recovers quickly after exposure to stress. These characteristics allow administration of drugs in retentive dosage forms. Additionally, the molecules absorbed via the buccal route enter the internal jugular vein, and thence into the systemic circulation, bypassing hepatic first-pass metabolism, leading to improved bioavailability. Furthermore, buccal administration is convenient, painless, and easily accessible [48,116,117].

Problems faced with this route of administration include saliva flow as well as the multi-layered epithelial surface of the oral cavity [5,48]. As with oral insulin, nanoparticles (NP) have been shown to facilitate buccal administration [5]. Absorption enhancers (e.g. bile acid salts, sodium lauryl sulphate, etc.) can aid buccal delivery by

increasing permeability of the buccal epithelium, or by inhibiting enzymatic action to improve stability of drug molecules. However, these enhancers present additional side effects, such as mucosal irritation as well as having an offensive taste. In particular, bile acid salts, one of the most effective enhancers, have a bitter taste [48].

Insulin-coated NP embedded in polymeric films showed promising results in enhancing insulin transport through a cultured tri-dimensional model of human buccal mucosa (EpiOral). The cationic polymer used is a polymethacrylate derivative, known as trimethylammonioethyl methacrylate chloride, Eudragit® RLPO (ERL) [46]. Another formulation recently studied involved insulin-loaded NP embedded into chitosan films. This preparation improved insulin absorption through the same EpiOral buccal mucosa model by 1.8 times, compared to pure insulin [47].

Vesicles can be utilised to improve insulin delivery through the buccal route. A new form of deformable lipid vesicle known as a transferosome was found to improve buccal absorption of insulin in rabbits. These transferosomes consist of soybean phosphatidylcholine, cholesterol, and sodium deoxycholate (NaDC). Experimental results indicate relative pharmacological activity and relative bioavailability of 15.59% and 19.78%, respectively, compared to SC administration of insulin solution. Transferosome delivery of insulin was also found to be better than delivery by conventional insulin vesicles [48,50].

In addition, many studies have tested the effect of absorption enhancers to improve buccal insulin delivery. One such formulation is a combination of soybean lecithin and propanediol, which was shown to enhance buccal insulin delivery *in vivo*, with significant hypoglycaemia lasting 4-5 hours [5,48,51,118]. Another experiment studied a Pluronic F-127 (PF-127) gel containing unsaturated fatty acids. Three types of fatty acids were tested, namely oleic, eicosapentaenoic and docosahexaenoic acids, all of which improved insulin release, caused continuous hypoglycaemic effects in normal rats, with oleic acid giving the highest pharmacological availability of $15.9 \pm 7.9\%$ [48,52]. Another compound, lysalbinic acid is also known to increase the permeability of buccal mucosa to certain peptides including insulin, and studies on hamster cheek pouch models have proven this [48,53,54].

Mucoadhesive delivery systems allow improved buccal absorption without the side effects associated with absorption enhancers. These formulations result in interactions between the delivery system and the mucosa, increasing the residence time of drug molecules at the absorption sites [48,119]. NP with mucoadhesive properties were also studied for buccal insulin delivery. These pelleted bioadhesive polymeric NP containing insulin resulted in significant hypoglycaemia in diabetic rats after 7 hours, with no risk of hypoglycaemia [48,49].

Oral-lyn™ is a market available buccal insulin preparation developed by Genex Biotechnology Corp. for patients with T1DM and T2DM. It is delivered via the company's RapidMist™ hand-held device, which allows buccal absorption and prevention of deposition in the lungs. Oral-lyn™ consists of a mixed micellar solution containing insulin and absorption enhancers. Each puff delivers 10U insulin, and with an absorption rate of 10%, results in 1 U of insulin absorbed [5,44,45,48]. Clinical trials involving individuals with T1DM, T2DM, as well as healthy subjects found that Oral-lyn™ showed a dose-dependent absorption profile, with faster onset and shorter duration relative to SC regular insulin [5,45].

Pulmonary (inhaled) insulin: Inhaled drugs are absorbed into the alveolar-capillary network, which has the advantage of having a large surface area (~140m²), thin diffusion barrier (~0.1-0.5mm), as well as being non-invasive. Drugs can be aerosolised and delivered with a median aerodynamic diameter smaller than 5µm, effectively targeting the alveoli as sites for absorption. Pulmonary delivery bypasses digestive enzymes and first-pass metabolism associated with oral delivery. Although enzymes are present in the lungs, they are involved in different metabolic pathways than those in the GIT [48,63,120].

Insulin microcrystals with a mean diameter of 3µm were prepared and administered to STZ-induced diabetic rats by using a sieve-type ultrasonic nebuliser. Prolonged hypoglycaemic effects were observed over 7 hours. This may be explained by a sustained-release profile of insulin from the microcrystals that were deposited throughout the lungs [48,57]. Further studies on insulin microcrystals found that the addition of zinc caused an enhancement of the hypoglycaemic effect in rats (17% minimum reductions in blood glucose) [48,56].

Hyaluronic acid (HA) exhibits valuable properties to aid in pulmonary insulin delivery, including its mucoadhesivity, which prolongs contact of the drug delivery system with absorption sites in the lungs, improving insulin absorption. *In vivo* studies on beagle dogs suggested that the addition of zinc ions (Zn²⁺) or hydroxypropyl cellulose (HPC) to insulin/HA dry powder system improved the mean residence time of the preparation by >9 times and >7 times, respectively, as compared to spray dried pure insulin [48,58].

A number of absorption enhancers have been experimented upon, to improve insulin delivery via the pulmonary route. *In vitro* and *in vivo* studies suggested that phospholipids enhance pulmonary absorption of insulin. When 1,2-dipalmitoyl phosphatidylcholine (DPPC) was physically mixed with insulin and administered to anaesthetised rats, greater hypoglycaemic effects were seen compared to insulin and liposome mixture [48,59]. Another study investigated the effect of additives to insulin absorption. A dry powder form of insulin was found to have a higher bioavailability than a pH 7.4 insulin solution. Bacitracin (1.4mg/dose) and Span 85 (1mg/dose) improved insulin (solution form) bioavailability to almost 100%, but are not effective in dry powder forms. However, citric acid added to insulin dry powder increased the hypoglycaemic effect, with bioavailabilities of 42% and 53% for dry powders containing 0.025 and 0.036mg/dose citric acid, respectively. Moreover, citric acid was not found to cause acute toxicity to lung cells [48,60]. Another group of absorption enhancers belong to the family of cyclodextrin (CD) derivatives, namely tetradecyl-β-maltoside (TDM) and dimethyl-β-cyclodextrin (DMβCD), which have been tested on anaesthetised rats. The relative bioavailability of insulin preparations with TDM was higher (0.34-0.84%) than those containing DMβCD (0.19-0.48%), indicating that TDM is a more effective absorption enhancer. It was also found that both agents have reversible effects on the respiratory epithelium, which was restored 120 minutes post-exposure [48,61]. Hydroxy-methyl-amino-propionic acid (H-MAP) was investigated as a potential pulmonary absorption enhancer in fasted anaesthetised rats. It was discovered that co-administration of H-MAP with insulin affects pharmacokinetics and pharmacodynamics in a dose-related manner, with maximum effects at 16mg/kg H-MAP and 1.3U/kg insulin. At these doses, there was a >2.5-fold increase in relative insulin bioavailability, achieving twice the maximum insulin concentration, with twice the reduction of blood glucose, compared to the same dose of insulin alone [62].

Liposomes are another method to improve pulmonary transport of insulin. Studies have found that liposomes can be made and insulin encapsulated (and remained stable) within them. *In vivo* studies in alloxan-induced diabetic mice indicated that aerosolised insulin-liposomes were homogeneously distributed throughout the alveoli and drug retention times were increased, causing a measurable hypoglycaemic effect [48,63]. Another particulate formulation combines calcium phosphate (CAP) and polyethylene glycol (PEG) to make suspensions of insulin-CAP-PEG particles. Pharmacokinetic analyses indicated that insulin had a longer half-life and residence time when given to fasted rats in CAP-PEG particles compared to in solution. Spray instillation was also found to be more efficient than intratracheal instillation to deliver insulin method via the pulmonary route [48,64].

Nanoparticulate delivery systems have helped improve pulmonary insulin delivery as well. One such formulation involves insulin-loaded poly(lactide-co-glycolide) (PLGA) nanospheres with a mean diameter of 400nm, prepared by modified emulsion solvent diffusion method in water. These NP were prepared as aqueous dispersions (6mg/ml),

nebulised by a sieve type ultrasonic nebuliser, and administered to fasted guinea pigs through a spacer by using a constant volume respirator for 20 minutes. Administration of 3.9IU/kg insulin caused substantial prolonged hypoglycaemic responses over 48 hours, in contrast to 6 hours by nebulised aqueous insulin solution. This suggested that insulin was released in a sustained manner from PLGA nanospheres that were distributed throughout the lung [48,65]. Insulin loaded in polybutylcyanoacrylate NP prepared by emulsion polymerisation also improved bioavailability in studies on normal rats. Intratracheal administration caused significant hypoglycaemic responses, with minimum blood glucose concentrations of 46.9%, 30.4% and 13.6% of initial levels after administration of 5, 10 and 20 IU/kg insulin (in polybutylcyanoacrylate NP), respectively [48,66].

A recently FDA-approved product called Afrezza® has been developed by MannKind Corp. This rapid-acting insulin is delivered as an inhalational powder before meals. Studies to assess its safety and effectiveness were carried out in 3017 participants (1026 with T1DM, 1991 with T2DM). When Afrezza® was used as part of the basal-bolus regimen with basal insulin in T1DM patients, Hb_{A1C} reduction in 24 weeks was acceptable but not as much as that achieved by insulin as part. When used in combination with oral hypoglycaemic drugs in T2DM patients, Afrezza® achieved a greater reduction in Hb_{A1C} after 24 weeks (compared to placebo) [55].

The ideal pulmonary delivery system would have good insulin-loading efficiency, avoidance of insulin break down, predictable and reproducible release profile, as well as minimal side effects. These are the challenges faced by researchers searching for an inhaled form of insulin therapy. It is important to consider the adverse effects of inhaled insulin including the greater risk of hypoglycaemia, greater weight gain, and mild to moderate cough in 25% of patients [48,121-123]. Insulin doses are also dependent on patient co-morbidities, as smokers and asthmatics require lower and higher doses, respectively [48,124,125]. Furthermore, to achieve a comparable glycaemic response, inhaled insulin needs to be given at much higher doses compared to SC administration [48,126,127]. There is also the issue of insulin-directed antibodies [48,128]. Nevertheless, inhaled insulin appears to be a reasonable alternative to short-acting insulin injections in diabetic patients [48,126,129].

Nasal insulin: The nasal cavity is lined by an epithelial layer that has a large surface area due to the existence of microvilli on the epithelial cells. Together with the high total blood flow and porous endothelial membrane, this facilitates absorption of molecules into the subepithelial capillary beds, directly into the general circulation. This route also bypasses the liver and avoids first-pass metabolism. All together, these can allow fast absorption (comparable to intramuscular or even IV injections) and onset at lower doses, and fewer side effects. Nasal administration is also convenient and less invasive, which can lead to better patient compliance [48,76,130,131]. Administering intranasal insulin post-meals result in pulsatile increases in blood insulin levels, mimicking normal physiological secretion. Considering all these advantages, intranasal insulin has great potential to be used in the treatment of patients with T1DM and T2DM [48,130].

Difficulties faced by nasal drug delivery include mucociliary clearance, enzymatic activity, as well as the epithelial lining itself that prevents passage of peptide molecules with high molecular weights and hydrophilic natures. Nasal bioavailability can be increased by use of absorption enhancers, proteolytic enzyme inhibitors, mucoadhesive formulations, as well as dry powder systems to deliver insulin via the nasal route [48,132].

One study investigated the potential use of alkylglycosides as absorption enhancers for the nasal absorption of insulin, as *in vivo* studies in rats made hyperglycemic by xylazine/ketamine anesthesia. When drops containing 2U insulin and 0.03-0.5% alkylglycoside were instilled, rapid hypoglycaemic effects were observed, together with increased levels of immune reactive insulin in the blood, with maximal hypoglycaemia between 60-120min post-administration.

Dodecylmaltoside, tridecylmaltoside, tetradecylmaltoside and dodecylsucrose (a compound that differs from dodecylmaltoside by only one carbohydrate residue) effectively enhanced insulin absorption. However, nonylglucoside needed higher concentrations of 0.25-0.5% to behave as an effective absorption enhancer [68]. Further experiments on glycosides, made up to maltose or sucrose linked to longer alkyl side-chains (C_{13-16}), were tested on anaesthetised rats, and their efficacy as absorption enhancers were compared with previously tested alkylglycosides with shorter alkyl side-chains (C_{8-12}). Results indicate that for alkylmaltoside derivatives, tetradecylmaltoside (C_{14}) caused maximal enhancement in insulin absorption. Longer-chained pentadecylmaltoside (C_{15}) and hexadecylmaltoside (C_{16}) were less potent and required higher concentrations for maximal effect. On the other hand, for alkanoylsucrose derivatives, tridecanoylsucrose (C_{13}) and tetradecanoyl sucrose (C_{14}) were most potent. In addition, tetradecyl maltoside increased insulin absorption even when administered 15 minutes before insulin. It was determined that the potency of the enhancers depended more on the alkyl side-chain length rather than the glycoside moiety [48,67].

Emollients such as sucrose cocoate (SL-40), which is a mixture of sucrose esters of coconut fatty acids in aqueous ethanol solution, has also been tested as a absorption enhancer for nasal insulin administration *in vivo* on anaesthetised Sprague-Dawley rats. A 0.5% sucrose cocoate formulation increased nasal insulin absorption, with resulting hypoglycaemic effects. Mass spectrometry identified sucrose monododecanoate as the most common ester in the sucrose cocoate mixture [69].

Mucoadhesive delivery systems can aid in improving bioavailability by countering the nasal mucociliary clearance mechanism. These systems prolong drug residence times at absorption sites and protect the drug molecules from enzymatic degradation [48,133,134]. These include gels, liposomes and microspheres, which absorb water from the mucus layer lining the nasal cavity and forming a gel-like layer over the nasal mucosa. In addition, the absorption of water causes the nasal epithelial cells to be dehydrated, resulting in the separation of tight junctions between cells, increasing their permeability to drugs via paracellular absorption [48,73,135]. An example of a gel preparation is a carbopol-based nasal gel spray, containing insulin, which caused a significant drop in blood glucose levels in rabbits. The bioavailability of insulin administered in the nasal gel preparation was 20.6% compared to IV insulin [48,70]. Another insulin gel formulation uses bioadhesive chitosan (CS) gel containing 4000 IU/dl insulin, administered to diabetic rabbits. Results show that gels made up of 2% medium molecular weight CS with ethylenediaminetetraacetic acid (EDTA) enhanced insulin absorption and caused a drop in glucose levels by up to 46% of that caused by IV insulin [48,71].

Microsphere preparations for nasal insulin include insulin/CS microspheres made by emulsification-cross linking process. *In vivo* studies on diabetic rats found that microspheres with 400mg CS and 70mg ascorbylpalmitate (used as a cross-linker) resulted in an absolute bioavailability of 44%, with a corresponding 67% decrease in blood glucose compared to IV insulin [48,72]. A separate study tested aminated gelatin microspheres (AGMS) to deliver intranasal insulin to rats. Fluorescein isothiocyanate (FITC)-labelled insulin was used in this study, which was released slower from AGMS (cumulative release of 18.4% within 30min, 56.9% within 8h), compared to native gelatin microspheres (GMS) (cumulative release of 32.4% within 30min, 75.1% within 8h). Additionally, AGMS increased nasal insulin absorption significantly when given in dry powder form. It was suggested that AGMS works by absorbing water from the nasal mucosa, temporarily dehydrating the epithelium, hence, opening tight junctions. The cationic nature and mucoadhesivity of AGMS also contributes to its absorption enhancing effects [48,73]. A separate microparticle-based delivery system using a thiolated chitosan conjugate, known as chitosan-4-thiobutylamidine (CS-TBA) was tested to deliver insulin nasally in rats. The nasal preparation consisted of FITC-labelled insulin, CS-TBA and reduced glutathione

(as a permeation mediator). The insulin showed a controlled-release profile over 6 hours, with an absolute bioavailability of 7.24%, which is higher compared to using unmodified CS [48,74,75].

Nanotechnology has opened up new pathways in researching novel nasal insulin delivery systems. One formulation tested insulin/CS NP prepared by ionotropic gelation of CS glutamate and tripolyphosphatepentasodium, and by simple complexation of insulin and CS, but found that the enhancing effect was not as great as CS in powder form [48,136]. Another formulation involves a CS derivative: PEGylated trimethyl CS (TMC) nanocomplexes (NC). These insulin/PEGylated TMC NC managed to reduce blood glucose levels by 34-47% in anaesthetised rats, and were found to have less toxic effects on nasal epithelium compared to the non-PEGylated counterparts, suggesting its suitability to be used as a nasal insulin delivery system [76]. Another set of NC tested for this same purpose were made from amine-modified poly(vinyl alcohol)-graft-poly(L-lactide), and prepared by spontaneous self-assembly of insulin and the water-soluble amphiphilic polymer. *In vivo* studies on fasted healthy rats, found that the NC decreased blood glucose levels by 50% (from basal levels), 50 to 80 minutes post-administration, and studies on STZ-induced diabetic rats observed a 30% decrease in blood glucose within 75-95 minutes. There was no evidence of nasal mucosal damage 4 hours post-administration, as confirmed by histological examination [77]. An amphiphilic glycopolymer poly(2-lactobionamidoethyl methacrylate-random-3-acrylamidophenylboronic acid) (p(LAMA-r-AAPBA)) was used in the manufacture of NP to deliver insulin intranasally. It was demonstrated that modification of the glycopolymers affects insulin release *in vitro*. Further tests show good cytocompatibility, with NP internalisation into Calu-3 cells via clathrin-mediated and lipid raft/caveolae-mediated endocytosis. *In vivo* studies on diabetic rats show significant hypoglycaemic effect after nasal administration [78].

Nasally delivered insulin seems to be a possible non-invasive method of administering insulin. However, there are certain problems that need to be addressed. There are large amounts of intra- and inter-individual variability in bioavailability, as well as limitations to the amount of insulin that can be administered for each nasal instillation. More importantly, the preparation must be safe to be used in the long run, as insulin is used in long-term diabetic management. Nasal irritation and possible damage to the nasal mucosa and ciliary function are concerning drawbacks [48]. Absorption enhancers are useful to increase nasal insulin bioavailability, but are also associated with adverse effects, especially at effective concentrations and/or prolonged use. Surfactants and bile salts, popular absorption enhancers, can cause irreversible nasal mucosa damage [48,137-139]. Another potent enhancer is dimethyl- β -cyclodextrin (DM β CD), a cyclodextrin derivative, which was shown to increase insulin absorption via the nasal route in rats at a concentration of 5% (w/v), with a bioavailability of almost 100%. However, *in vitro* studies show that DM β CD affects ciliary movement [139]. Hence, further studies are needed before nasal insulin delivery systems are ready for clinical use.

Ocular insulin: Insulin can also be made into eye drops to allow non-invasive administration. This route is also relatively convenient and allows rapid systemic absorption, bypassing the GIT and liver. Moreover, tissues in the eye are less likely to develop immunological reactions, compared to other tissues. This is supported by a 3-month study on subjects that was administered daily insulin eye drops, and found no ocular side effects or tolerance [48,140]. Problems with this route of delivery are local irritation, as well as loss of drug molecules via blinking, tearing and drainage [48]. Insulin delivered as eye drops in a randomised, placebo-controlled trial was shown to not cause any clinically detectable toxic effects to the anterior eye structures in healthy human volunteers (in single doses up to 100 U/mL). However, results of this study also indicated that there was no significant systemic absorption of insulin without any effect to blood glucose levels [48,141]. A separate study on rats also showed insignificant insulin absorption when applied as eye drops as a solution in normal

saline. When emulsants were added (e.g. saponin, Brij-78, BL-9, and several alkylglycosides), insulin absorption improved and hypoglycaemia was observed [142].

Due to the limited bioavailability of insulin delivered through the ocular route, numerous absorption enhancers have been experimented upon. Fusidic acid and glycocholate were proven to increase ocular insulin absorption in rabbits, especially when administered at higher pH levels (pH 8 as opposed to pH 3.5) [81]. In a study on albino rabbits, 1% concentrations of polyoxyethylene-9-lauryl ether (POELE or BL-9), sodium glycocholate (NaGC), sodium taurocholate (NaTC), and sodium deoxycholate (NaDC) were each tested as absorption enhancers, and it was found that hypoglycaemia was possible with ocular insulin preparations. The bioavailabilities ranged from 3.6-12.6% with absorption enhancers, as compared to 0.7-1.3% without enhancers. However, systemic absorption of insulin was contributed by the nasal mucosa more than by the conjunctival mucosa by about 4 times [48,79].

Alkylglycosides have also been shown to enhance systemic absorption of insulin delivered via ocular delivery. Studies on rats show that tetradecyl-, tridecyl- and dodecylmaltoside as well as dodecylsucrose showed effective improvements in insulin absorption, at concentrations as low as 0.125%, as compared to NaGC (a bile salt), which is only effective at concentrations over 0.5%. The best absorption enhancers were the alkylglycosides with the greatest hydrophobic properties [48,80]. Studies on healthy dogs and cats have also been carried out to investigate a variety of absorption/permeation enhancers, namely saponin, POELE (BL-9), polyoxyethylene-20-stearyl ether (Brij-78), fusidic acid, dodecylmaltoside, and tetradecylmaltoside [48,82,83]. In dogs, 0.5% saponin, 0.5% and 1% POELE (BL-9), 0.5% and 1% dodecylmaltoside, as well as 0.5% and 1% tetradecylmaltoside significantly enhanced insulin absorption with corresponding glycaemic effects [48,83]. *In vivo* studies in cats showed that substantial increase in insulin absorption and hypoglycaemic effects were noted when insulin was administered with 0.5% saponin, 0.5% Brij-78, as well as 1% and 2% POELE (BL-9) [82]. In contrast, ocular delivery of native insulin had no significant effects in both dogs and cats, insulin delivered with 0.5% Brij-78 and 0.5% fusidic acid had no significant effects in dogs, and insulin delivered with Brij-99 had no significant effects on cats [82,83].

In a separate study, insulin-loaded cationic liposomes were prepared to deliver and prolong insulin retention time in the precorneal area of the eye, which produced hypoglycaemic effects in rabbits [48,84,143]. Liposomes made up of egg phosphatidylcholine with cholesterol and stearylamine (10:2:1 in weight ratio), loaded with insulin, resulted in significant hypoglycaemic effects in normal rabbits 90-120 minutes post-administration [84].

Absorption enhancers may cause adverse effects; hence, these compounds were also tested for possible unwanted reactions in test subjects. Saponin (0.5% conc.) caused temporary irritation to the eyes, whereas Brij-78 and POELE (BL-9) were well tolerated in studies on dogs and cats [82,83]. Fusidic acid, dodecylmaltoside, and tetradecylmaltoside showed no side effects in dogs [83]. *In vivo* studies on POELE (BL-9) and Brij-78 on rabbits as permeation enhancers for ocular insulin administration, showed no local side effects or allergic response after 3 months of twice daily ocular instillations. Uptake and efficacy of insulin also remained constant throughout the study period [48,144].

Gelfoam®, a commercially available absorbable gelatin sponge, has been used in studies to fabricate an ocular device for the delivery of insulin. *In vivo* studies achieved prolonged insulin delivery, especially with the addition of Brij-78 as an absorption enhancer. There were no risk of hypoglycaemia, and near normal glucose levels were achieved within 60 minutes after the removal of the insert [48,85,86]. Due to the potential side effects of absorption enhancers, the role of acid in insulin delivery via Gelfoam® devices, without absorption enhancers was investigated. Results suggest treatment of Gelfoam® devices with

either 5% acetic acid or 1% hydrochloric acid (HCl) was sufficient to improve systemic absorption of insulin [48,87-89]. *In vivo* studies on rabbits show that 0.2mg of insulin in a single Gelfoam® device managed to lower blood glucose levels to 60% of initial levels over 8 hours, and no eye irritation was caused by Gelfoam® devices treated with acetic acid up to 30% concentration [89].

Insulin delivered via the ocular route seems possible with no significant toxicity, as suggested by preliminary studies. However, it is important to note that intraocular devices presents with a variety of complications, partly due to the fact that it needs to remain in place for a few hours. This makes local irritation effects more problematic [48]. It has also been suggested that insulin might have metabolic and/or mitogenic effects on the ocular surface in humans [145]. Comfort is another issue, as well as ensuring the device does not get dislodged during daily activities as well as during sleep. Finally, it is important that the release of insulin from ocular devices is constant and reproducible. As of yet, further studies need to be carried out before ensuring that ocular insulin can be used clinically in diabetic patients [48].

Rectal insulin: This route involves absorption in the GIT, but differs from the oral route as it bypasses the more proximal areas that are actively involved in digestion, as well as being relatively unaffected by diet and gastric/intestinal motility. Hepatic first-pass metabolism may also be avoided to a certain extent as there are porto-systemic anastomoses in rectal vessels. These vessels connect the portal system to the systemic system, hence allowing absorbed drugs to directly enter the systemic circulation [48].

In animal studies, suppositories were formulated with 50 U insulin and either 50mg deoxycholic acid (DCA), sodium taurocholate (NaTC), or both, and tested on alloxan-induced hyperglycemic rabbits. These resulted in relative hypoglycaemic effects of 38.0%, 34.9%, and 44.4%, respectively, compared to SC insulin (40 U). When 50mg polycarbophil was added to the preparation containing insulin, DCA and NaTC, the relative hypoglycaemia increased to 56%. Therefore, approximately 50% of the efficacy of SC insulin can be achieved with rectal insulin formulations [48,90]. Studies on diabetic beagle dogs found that 50mg sodium salicylate or 1% POELE (BL-9) enhanced insulin absorption from suppositories (containing 5 U /kg insulin and Witepsol W35 as a base), producing a relative hypoglycaemic effect of approximately 49-55% (compared to SC insulin 4 U/kg) [48,91]. Similar hypoglycaemic effects (approximately 50% relative to SC insulin) were achieved with insulin-loaded Witepsol W35-based suppositories containing either sodium deoxycholate (NaDC) plus sodium cholate (NaC) (100mg/50mg, respectively), sodium taurodeoxycholate (NaTDC) (100mg), or sodium taurocholate (NaTC) (100 mg) as rectal absorption enhancers. Adjusting the insulin dose in the rectal formulation in accordance to the degree of hyperglycaemia allows a desirable hypoglycaemic effect [48,92]. Gel formulations have also been tested for transrectal insulin delivery. A study on rabbits utilised CS gel as a carrier, with dimethyl- β -cyclodextrin (DM β CD) as absorption enhancers. Prolonged insulin release was achieved by the CS gel, and maximum hypoglycaemic effects were seen in a formulation containing insulin-CS gel with 5% DM β CD. This study found that such a delivery system is able to improve insulin delivery transvaginally as well [93].

Suppositories containing 100U insulin and 200mg sodium salicylate as an absorption enhancer were tested in human studies (4 healthy, 15 T1DM individuals). Hypoglycaemic effects were achieved by 15 minutes, and lasted up to 90 minutes post-administration [94]. Another human study experimented on insulin suppositories containing 200U insulin with 100mg sodium cholate (NaC), which produced a maximum of 47.7% reduction in blood glucose levels (90min post-administration) compared to 50.6% with SC insulin (20U). This preparation managed to avoid significant postprandial rise in blood glucose in 7 T1DM patients, and were found to be safe and well-tolerated. However, when tested on healthy control subjects, it caused severe hypoglycaemia 1 hour post-administration [95].

An additional technique to improve rectal absorption of insulin is by creating an adhesive interaction between the delivery system and the rectal mucosa, increasing drug residence time at the absorption sites. This mucoadhesive concept has been tested with a thermo-reversible insulin liquid suppository, which at body temperature, undergoes a phase transition to form bioadhesive gels. Insulin bioavailability was improved with this formulation, proven by *in vivo* experiments on STZ-induced diabetic rats. The suppositories consist of 100 IU/g insulin, 15% poloxamer P407, 20% poloxamer P188, 0.2% polycarbophil, and 10% sodium salicylate. The liquid suppositories were easy to deliver, painless, safe, and remained at administered sites [48,96]. Another mucoadhesive preparation utilises snail mucin motifs extracted from the giant African snail *Archachatina marginata*. These mucin motifs (molecular weight 5780Da) contain cysteine residues that form disulphide linkages with cysteine residues in endogenous mucin, leading to sustained release of co-administered insulin. Results indicate that 7% mucin caused 66% reduction of blood glucose levels in rats within 2 hours of administration of the glycerol-gelatin suppository loaded mucin [48,97].

Rectally delivered insulin seems to be a possible alternative for less invasive insulin therapy. Nevertheless, insulin administered through this route appears to have lower bioavailability and efficacy, compared to SC forms, as indicated by experimental results to date. The incorporation of absorption enhancers as well as modification of the suppository formulation to incorporate mucoadhesivity opens up possibilities to improve the delivery of transrectal insulin. However, for long-term therapy, it is worth considering the potential adverse effects, such as increased permeability of rectal mucosa to toxic substances present in the GIT lumen, which may be an unwanted result of using absorption enhancers. Furthermore, rectal delivery is not the most convenient method of administering medications due to the associated discomfort, and suppositories often require special storage conditions. Thus, the improvement in patient compliance from replacing SC insulin with rectal insulin may not be significant [48].

Transdermal insulin

Another method to deliver insulin into the bloodstream in a non-invasive manner is through the skin surface into the underlying capillary network. This method of delivery is convenient, which would lead to better patient compliance. However, the stratum corneum layer on the outermost surface of the skin acts as the main barrier for transdermal delivery, only allowing small lipophilic molecules to be absorbed. Many methods have been developed to overcome this barrier, some of which include chemical absorption enhancers, iontophoresis, sonophoresis/phonophoresis, electroporation, use of microneedles, and laser ablation. Additionally, new drug carriers can also aid transdermal insulin transport (e.g. nano- and microparticles, lipid vesicles) [5,48,146-149].

A study involving insulin-loaded micro-emulsions has shown promising results in achieving transdermal insulin transport through goat skin. Insulin was incorporated into micro-emulsions using oleic acid as the oil phase, Tween 80 as the surfactant, and isopropyl alcohol as the co-surfactant. It was found that the formulation with 10% oleic acid (38% aqueous phase, 50% surfactant phase) with 2% dimethyl sulfoxide (DMSO) as a permeation enhancer, showed maximum permeation flux through goat skin [98]. Another potential formulation for transdermal insulin delivery is via transferosomal gels containing insulin, which showed transdermal absorption with zero-order kinetics through porcine ear skin, as well as prolonged hypoglycaemic effects (over 24 hours) in diabetic rats [99]. A recent study explored the use of amidated pectin hydrogel matrix patches to deliver insulin to diabetic rats, which proved that this system managed to deliver physiologically relevant amounts of insulin, and that the delivered insulin retained pharmacological activity [100]. Insulin emulgel has also been tested for transdermal delivery. The emulgel was prepared using a combination of carbomer or hydroxypropyl methylcellulose (HPMC) as gelling agents and polysorbate 80 as an emulsifier, with emu oil as a penetration enhancer. Albino rabbits

treated with the insulin emulgel alone experienced a drop in blood glucose from initial value of 250(\pm 10) to 185(\pm 7)mg/dl at 120 minutes post-administration. Adding iontophoresis caused a greater hypoglycaemic effect (glucose levels decreased to 125(\pm 5)mg/dl at 120 minutes) [101].

Although a potential non-invasive route for effective insulin therapy, transdermal insulin delivery requires further investigation, including clinical studies, to improve insulin bioavailability, as well as ensuring safety of the drug preparation. This is particularly important for the skin, which is more at risk of adverse immune reactions [48].

Conclusions

These advancements in insulin therapy provide opportunities to overcome the disadvantages of current insulin regimens. Ultra-long acting insulin (e.g. degludec) may lead to a convenient reduction in dose frequency, whereas ultra-short acting forms (e.g. Linjeta™) can avoid hypoglycaemic effects after meals. Artificial pancreas systems resemble physiological insulin, and with improved algorithms, have come ever closer to achieving physiological insulin levels and response to meals, especially when used in combination with new ultra-short acting insulin formulations. Transmucosal routes have shown promising results and may soon allow non-invasive insulin delivery for all diabetic patients. Finally, oral insulin seems to be the ideal formulation, which is being extensively researched by numerous companies and institutions worldwide. Nanotechnology has aided substantially in the improvement of insulin delivery as well.

However, it is wise to be cautious with exploring alternative and novel routes for insulin delivery, as insulin may produce unwanted local effects. It is well known that subcutaneous injections of insulin cause local lipodystrophy, damaging subcutaneous fat tissue and causing a degree of disfigurement [150]. Since insulin is a growth factor, it has the tendency to be mutagenic, which is a concern, as insulin therapy is usually life-long. Such prolonged exposure to a potentially mutagenic drug may adversely affect local tissues [6,10,151]. In addition to adverse effects inherent to insulin, the use of absorption enhancers may present their own problems. The barriers to absorption of drug molecules function to prevent penetration of toxins and pathogenic microorganisms. Disrupting such barriers may not only facilitate insulin absorption, but also transport of unwanted molecules and organisms [152]. In addition to that, hepatic first-pass metabolism may reduce bioavailability of insulin, but this process takes place in normal individuals to reduce hepatic glucose production as well as prevent peripheral hyperinsulinaemic effects [5,6,8-10]. Bypassing the liver to increase insulin bioavailability might cause adverse effects in the long run.

In conclusion, much work needs to be done to achieve a safe, non-invasive, convenient insulin formulation. With the release of Oral-lin™ and Afrezza®, and preliminary research in countless non-invasive insulin delivery systems, much progress has been made towards achieving this goal. Aided by the advancements in nanomedicine, the vision of an ideal insulin tablet may soon be realised, thus, making insulin injections a thing of the past.

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