Teaser Development of non-invasive insulin formulations for diabetes therapy has attracted a lot of research interest in the recent past. This article reviews the triumphs and tribulations of those in clinical trials and in commercial market.

A review of non-invasive insulin delivery systems for diabetes therapy in clinical trials over the past decade

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At present, the main form of insulin administration is the invasive subcutaneous (s.c.) route and, for many patients, this means managing their glucose levels with multiple daily injections, which is both painful and difficult to administer chronically. To increase patient compliance, products are slowly reaching the market that are more patient friendly, such as the insulin patch-pump systems, including Omnipod and V-Go, but also the inhaled-insulin Afrezza® and the buccal insulin Oral-lyn™. In this review, we outline the history of insulin, the various options that are currently available in practice for insulin delivery, and the non-invasive delivery systems that have entered the different stages of clinical trials over the past decade.

Introduction

Diabetes mellitus (DM) is a metabolic disease characterised by deficiency or development of resistance to insulin or, in some cases, both. In 2012, around 1.5 million deaths were caused by the disease and, according to the 2016 WHO Global report on DM, the estimated number of adults with the condition has increased considerably, from 108 million in 1980 to 422 million in 2014 [1]. It was predicted that a total of 366 million people would have DM by 2030, although, based on the International Diabetes Federation (IDF), this prediction had already been reached in 2011, and it was re-estimated that, by 2030, as many as 552 million adults would have the disease [2,3]. In the latest estimation, published in 2018 and shown in Table 1, the IDF estimated the number of adults (aged 18–99 years) with DM to be 451 million, in 2017, and the projection for 2045 is a substantial increase to 693 million [4].

Currently, for patients with insulin-dependent DM, the main route of insulin administration is parenteral. For individuals to maintain the correct level of blood glucose, this requires them, in many cases, to chronically administer multiple daily injections of either long-acting, intermediate-acting, short-acting, and/or rapid-acting insulin via the s.c. route. To increase patient compliance, and to reduce the burden of daily painful administrations of insulin, many researchers are working on the development of novel carrier systems for the safe and effective delivery of insulin via non-invasive routes, which mainly include buccal, oral, pulmonary, nasal, and transdermal systems. In this review, we summarise the options currently available for insulin

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TABLE 1

Estimated number and prevalence of adults with diabetes[a]

Year	Number (millions)	Prevalence of adults (%)
2017	451	8.4%
2045	693	9.9%

[a] From Ref. [4].

delivery, including Oral-lyn™ and Afrezza®, and then focus on non-invasive delivery formulations that have entered clinical trials over the past decade [5,6]. The information is primarily based on ClinicalTrials.gov, which is a database of clinical studies taking place worldwide and conducted with human participants [7].

Diabetes mellitus

The WHO classifies DM into: type 1 (T1DM), type 2 (T2DM), gestational diabetes, and intermediate conditions, such as impaired glucose tolerance and impaired fasting glycaemia, which can progress into diabetes [1]. The aetiologies of the two main types of DM (T1DM and T2DM) are complex and, based on current evidence, involve both genetic and environmental factors [8]. T1DM, also known as early-onset, insulin-dependent or juvenile DM, is caused by absolute deficiency of insulin as a result of autoimmune destruction of insulin-producing β cells in the pancreas. Approximately 10% or fewer of patients with DM are classified as having T1DM, and are diagnosed commonly during early childhood or as young adults. T2DM is the most common form of diabetes, also known as late-onset or non-insulin-dependent DM, which is responsible for almost all the other cases of DM and is results from insulin resistance and relative insulin deficiency.

In epidemiological studies, a fasting blood plasma glucose of ≥7 mmol/l or ≥126 mg/dl is accepted for diagnosis of DM as per the 1999 WHO criteria [9]. Uncontrolled DM in the short-term can result in dangerous complications, such as hypoglycaemia (<4 mmol/l), hyperosmolar hyperglycaemic state (often >40 mmol/l) and diabetic ketoacidosis. In the long-term, patients with DM often experience other medical problems, including cardiovascular disease, kidney failure, loss of vision, and neuropathy leading to possible leg amputation [1]. The Diabetes Control and Complications Trial (DCCT) demonstrated that intensive insulin therapy can delay microvascular complications, such as retinopathy and nephropathy in patients with T1DM, and that the benefits remain for at least 4 years thereafter [10–12]. By contrast, in clinical practice, one of the drawbacks of controlling blood glucose levels too strictly is the increased risk of hypoglycaemic episodes.

History of insulin

In 1923, 2 years after the discovery of insulin by Banting and Best, the company Eli Lilly, in partnership with the University of Toronto, produced large-scale purified animal insulin, which resulted in record profits for the company [13,14]. During the first 60 years following the discovery of insulin, optimisation efforts focused on isolating insulin from animal sources, primarily from bovine and porcine pancreata. However, in 1982, after regulatory approval of the world's first recombinant DNA drug, Humulin (human insulin), by the UK, USA, the then West Germany, and The Netherlands, the focus on purification processes shifted towards mainly enhancing properties of insulin through changes in both the formulation and amino acid composition [15]. In 1996, Eli Lilly and Co. developed the first commercial insulin analogue, insulin lispro (Humalog), by altering the naturally occurring amino acids in positions 28 and 29 of the B-chain to form Lys (B28) and Pro (B29) human insulin [15,16].

A variety of insulin types are now used in clinical practice, the amino acid sequences of which differ depending on whether the insulin is human insulin, bovine insulin, porcine insulin, or analogues of human insulin. However, bovine insulin is now rarely used because the main method of production is with recombinant DNA technology using bacteria and yeast [17]. After US Food and Drug Administration (FDA) approval of Pfizer's inhaled insulin product Exubera in 2006, even though the invention was unsuccessful, main research efforts now focus on producing alternative non-invasive formulations of insulin or analogues with transformed properties [18]. Two recent successful developments in the area, both developed by Novo Nordisk, include approval of the longer-acting insulin degludec (Tresiba) injections by the FDA and European Medicines Agency (EMA) and the most recent ultra-fast rapid-acting insulin aspart injections, known as Fiasp, approved by the EMA [19–21]. Fiasp is both faster and more stable compared with conventional insulin aspart (Novorapid® or NovoLog®) because of the addition of two extra excipients, nicotinamide and arginine, and, hence, it is suggested to result in insulin release more reflective of physiological insulin [22].

Structure, biosynthesis, and properties of insulin

The monomeric human insulin, with a molecular weight of 5.8 kDa, comprises 51 amino acids, and takes the form of an A chain of 21 amino acids and a B chain of 30 amino acids. As shown in Fig. 1, two disulfide bonds connect the A and B chains (A7–B7 and A20–B19) and one disulfide linkage is present within the A chain (A6–A11) [23,24]. Insulin in its primary form exists as a chain of 110 amino acids, known as preproinsulin, which is translated from insulin mRNA in the cytosol of pancreatic β cells [23,25]. There are several factors that control the biosynthesis of insulin, glucose metabolism being the central event, which results in the stimulation of insulin gene transcription and mRNA translation [26].

During the biosynthesis of insulin, the interaction of the signal peptide of preproinsulin with the cytosolic ribonucleoprotein signal recognition particle (SRP) facilitates the translocation of the molecule across the endoplasmic reticulum (ER) membrane to the luminal side of the rough ER [23,27,28]. In this process, the 24-amino acid signal peptide is cleaved off by signal peptidases, which results in the formation of the 86 amino acid-long proinsulin comprising the B chain, C peptide, and A chain [17,29,30]. Within the ER, proinsulin obtains the disulfide linkages, and the 3D structure required for its function.

Proinsulin transportation from the ER to the Golgi apparatus occurs via secretory vesicles and both GTP and calcium are required for the process [8,31]. As comprehensively reviewed by Dunn *et al.* [32], in the storage and/or secretory vesicles of the Golgi apparatus, proinsulin starts forming zinc- and calcium-containing dimers and then hexamers. Hence, the vesicles serve as storage sites for insulin and the C-peptide. In the secretory vesicles,

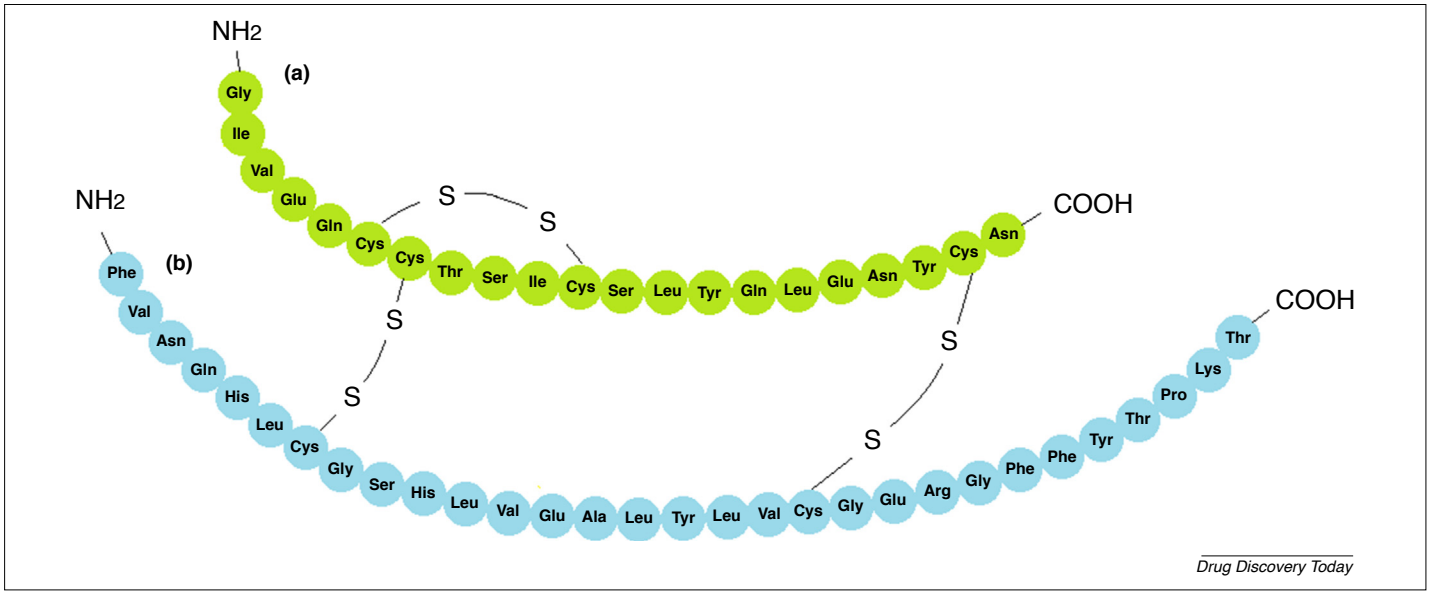

FIGURE 1

Schematic representation of human insulin, which comprises an A chain of 21 amino acids and a B chain of 30 amino acids. Two disulfide bonds connect the A and B chains (A7–B7 and A20–B19) and one disulfide linkage is present within the A chain (A6–A11) [23,24].

enzymes remove the C-peptides of proinsulin to produce mature insulin hexamers, which leads to crystallisation of the hexamers because mature insulin is less soluble compared with proinsulin [8,23,32]. Insulin secretion from the pancreatic β cells occurs when the plasma membranes of the storage vesicles fuse with the cell membrane in response to high plasma glucose levels. The release of the crystallised insulin hexamers from the vesicles in the intercellular space results in the crystals dissolving and releasing the biologically active insulin monomers [32].

Routes of insulin delivery

The aim of insulin therapy in patients with insulin-dependent DM, is to achieve plasma insulin levels that mimic as closely as possible the normal secretion of insulin in individuals without DM. This means covering both the release of basal or baseline insulin, typically in the range of 5–15 U/ml, and insulin released in response to meal intake, which generally results in peak serum insulin levels of 60–80 U/ml [17,33]. Since the original discovery of the hormone, the main method of insulin administration has been via s.c. injections. Figure 2 shows some of the various routes of insulin delivery currently in use and some routes of delivery that are under investigation in clinical trials and that will be discussed in this review.

Insulin therapy currently in clinical practice

Modern s.c. administration devices are safer, offer more accurate dosing, and have fewer complications compared with the original use of vials, which required patients to draw the prescribed doses of insulin from the vials using separate syringes. The innovation of the first pen injector, NovoPen® in 1985, was a success and now the main form of insulin administration is through the use of pen devices. Although the original pen device had to be refilled using disposable insulin cartridges, the injection pens that are now in more common practice are fully disposable, such as FlexPen® by Novo Nordisk and SoloStar® by Sanofi-Aventis [34]. The main advantages of s.c. insulin therapy include high bioavailability, relatively controlled onset of action, and flexibility in dosing. The disadvantages include the risk of lipodystrophy, painful administration, bruising of injection sites, weight gain, and alterations in absorption when the injected limb is used for strenuous exercise. In terms of time profile, the onset of action of short-acting insulins (i.e., soluble or regular insulins) is ~30–60 min, with maximal effect occurring at 2–4 h, and lasting for up to 8 h. Rapid-acting insulins, compared with regular insulin, are faster in onset and have a shorter duration of action. The intermediate-acting formulations, such as isophane human insulin (Humulin I), and long-acting preparations, such as insulin glargine (Lantus), have an onset of action of 1–2 h, peak levels are reached around 4–12 h, and the duration of action is 16–42 h [35].

Subcutaneous insulin patch-pump systems

Using a similar concept to the continuous s.c. insulin infusion (CSII) systems, companies are now developing more discreet and convenient devices, labelled as patch-pump systems, which do not involve the attachment of tubes [36]. Omnipod is such a device and is available in the USA, UK, Germany and Israel, among others [37]. The system comprises two parts: a small Pod, which is the insulin reservoir (200 U of insulin) and delivers insulin via a discreet needle; and a handheld Personal Diabetes Manager (PDM), which uses wireless communication to deliver basal insulin continuously. The Pod can provide up to 72 h of insulin [37].

One of the most simplistic patch systems is the V-Go™ device, designed by Valeritas, because it does not require batteries, programming, or a handheld remote [38]. The daily disposable V-Go™ insulin delivery device is designed to deliver a 24-h cover of both basal and bolus insulin and is suitable for patients being treated with multiple daily injections. The V-Go device is loaded by the patient with a U-100 rapid-acting insulin analogue, com-

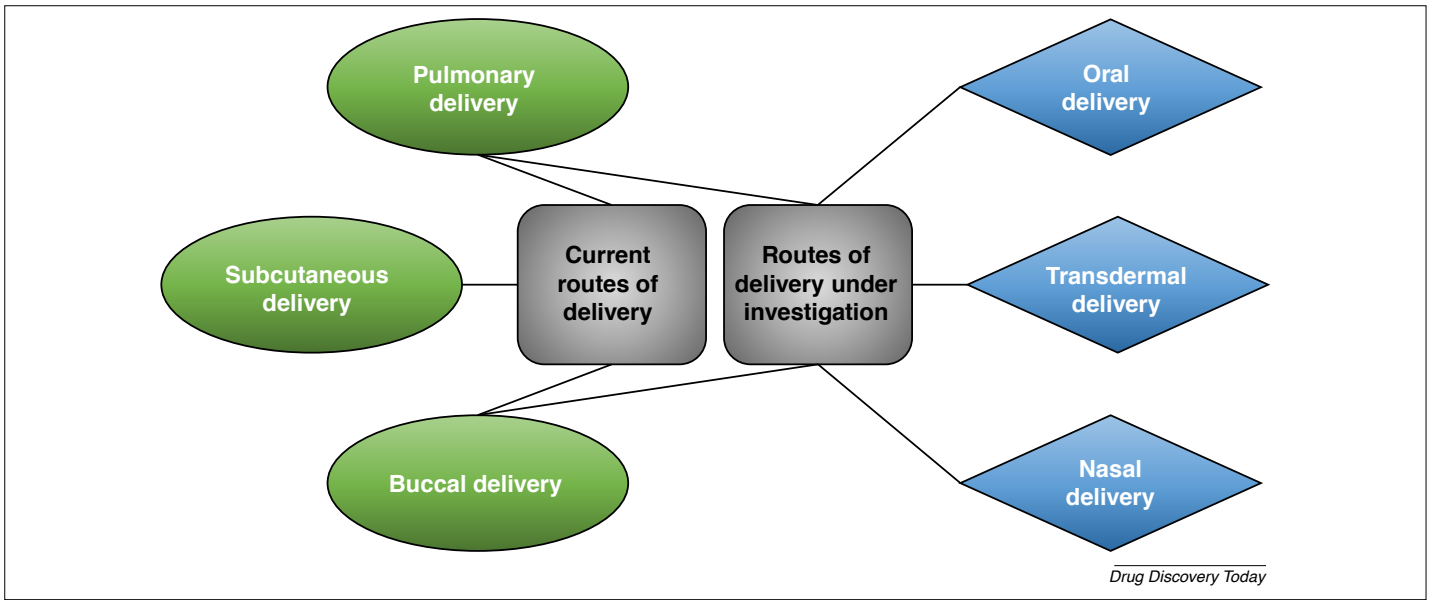

FIGURE 2

Routes of delivering insulin currently in use and routes of insulin delivery under investigation. The routes in the oval shapes represent the routes not only currently in use, but also in development (pulmonary, subcutaneous, and buccal delivery) and the routes in the diamond shapes are the main routes currently being trialled in humans (oral, transdermal, and nasal delivery).

patible with both Humalog (insulin lispro) and NovoLog (insulin aspart), using the adapter (EZ fill), which is provided with every 30-day supply of the devices. The device is small, lightweight (~25 g) and waterproof; after loading, the patient can attach the device comfortably and discreetly to the skin of their abdomen, arms, or thighs via a hypoallergenic adhesive strip [39]. To improve patient comfort, Floating Needle™ technology is used, which allows movement of the needle relative to the device. The push of a button allows the floating needle (30-gauge and 4.6 mm) to be inserted into the s.c. tissue, which starts the basal infusion of insulin for the full 24-h period. The basal rates are pre-set and are available as 20, 30, or 40 U of insulin per 24 h and each one also allows for 36 U of bolus insulin daily. The meal-time administration of insulin is initiated by the patient by two simple sequential clicks of the bolus-ready and bolus-delivery buttons. The device was launched by Valeritas in May 2012 [38].

Evaluation of insulin delivery via non-invasive routes

S.c. administration of insulin has some advantages; but because it is an invasive technique, alternative routes of administration are being investigated. Although there are other non-invasive routes, including vaginal, rectal, and ocular, Fig. 3 shows a summary of the advantages and disadvantages of the main routes under investigation, which are discussed further here.

Pulmonary insulin delivery

Of the inhaled insulin products that have reached clinical trials, only two products, both dry powder inhaler (DPI) systems, have secured FDA approval; one being Exubera, which also has EMA approval in Europe, and Afrezza® [18,49,50]. In 2007, the withdrawal of Exubera from the market by Pfizer led several other companies, such as Novo Nordisk and Eli Lilly, to discontinue the development of their inhaled insulin products around the same time [18]. As reviewed by Heinemann [18], Exubera was unsuccessful mainly because of the design of the device not taking into consideration the perspectives of patients. Although theoretically envisioned well for the purpose, in practice, the device was large and bulky and, thus, difficult for patients to carry around and could not be used with discretion. Other factors that contributed to the failure of Exubera included the unimpressive launch of the product, the training required to use the device, the high cost of the product, difficult dosing equivalence, the requirement for patients to regularly check their lung function, and safety concerns over chronic use of the product [18].

Nevertheless, another inhaled insulin product is currently in clinical trials (Table 2): the pocket-sized insulin inhaler device Dance-501, which uses a vibrating mesh micropump technology developed by Aerogen [50]. The device (Fig. 4) appears to have some potential for achieving pulmonary insulin delivery, based on the recent completion of Phase II clinical trials. The advantages of the Dance-501 is that it is small, discrete, portable, and battery operated and, hence, the developers have overcome the disadvantages faced by Exubera [50]. Additionally, because the formulation is a liquid aerosol system, the incidence of coughs is lower compared with DPI systems, and the price of using this device is said to be comparative to that of current s.c. pen devices [50]. One possible drawback of the design is that the insulin release is breath actuated, which, without good initial training, could result in fluctuating bioavailability, similar to current problems faced with such inhaler devices used for respiratory diseases. Another weakness is that, before administration, precise volumes of the formulation from a separate container are required to be dispensed into the inhaler reservoir, which adds another step to the administration process and the manual work required for the process could be a problem for older patients or those with arthritis. Although there are limited studies available concerning the bioavailability of the

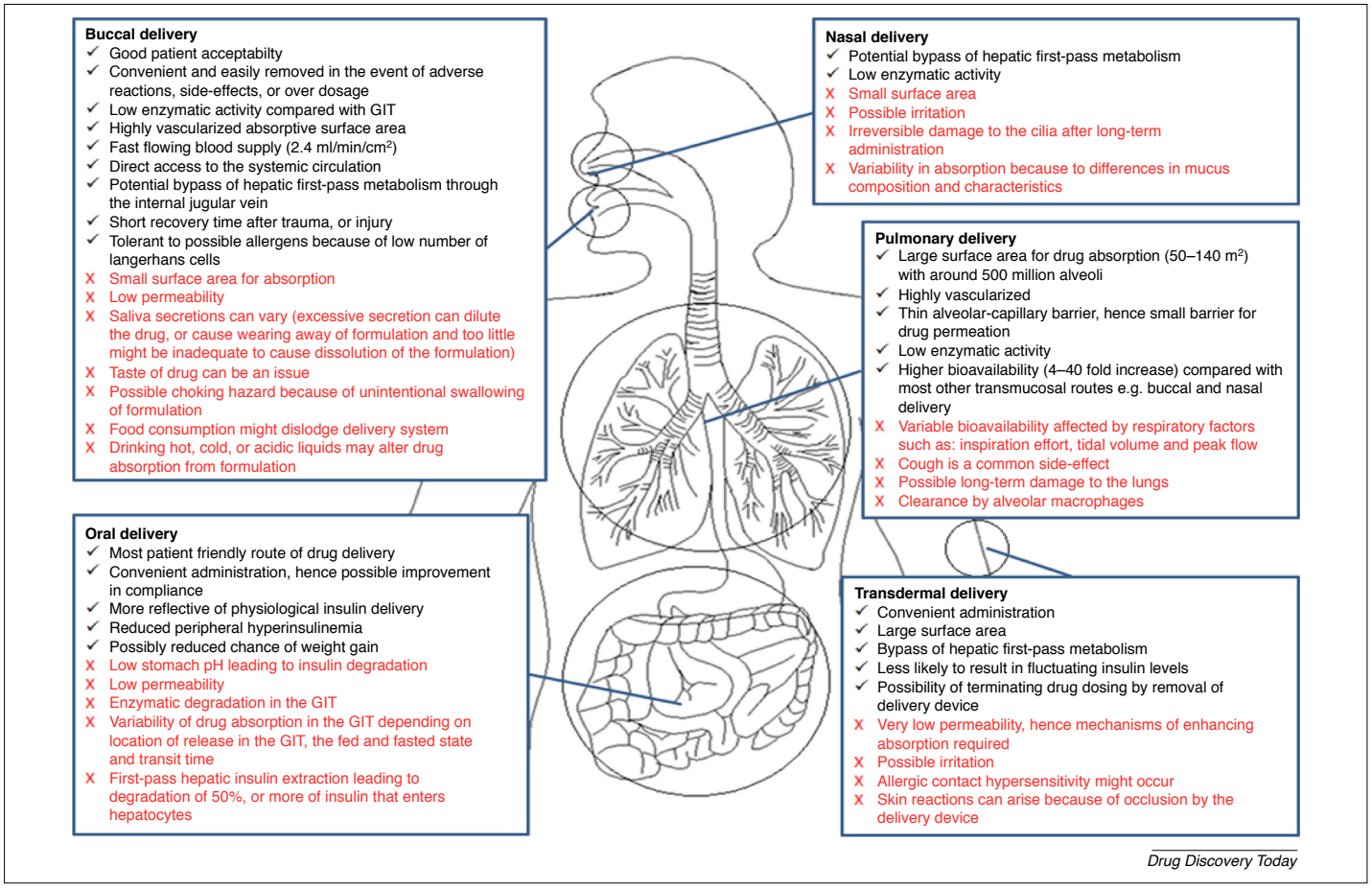

FIGURE 3

Summary of advantages and disadvantages of the five main non-invasive delivery routes (buccal, oral, nasal, pulmonary, and transdermal) for insulin delivery based on information from published reviews [40–48]. Abbreviation: GIT, gastrointestinal tract.

formulation for the Dance-501 device, a study completed with 24 patients with T2DM with a previous device, called Aerodose, which used Aerogen's electronic aerosol generator technology, showed no significant difference in the relative bioavailability and relative biopotency of inhaled insulin compared with s.c. administration of insulin among doses used at fixed ratios [51]. Aerodose was found to obtain overlapping dose–response curves with inhaled and s.c. administered insulin. The amount of inhaled insulin to be administered was required to be ten times greater than the s.c. dose (e.g., 160 U of inhaled insulin was approximately equivalent to 16 U administered using s.c. injections). The positive outcome from the study was also the fact that the inhaled formulation reached maximum serum insulin (T_{max}) concentration significantly ($P < 0.001$) faster compared with s.c. insulin administration, which could be explained by the high permeability across the thin alveolar–capillary barrier. Moreover, in terms of lung function, no serious adverse events or clinically relevant changes were observed. Overall, this could be a promising formulation for future use in the clinic.

Buccal insulin delivery

Apart from Generex Oral-lyn™, mentioned below, another interesting new buccal formulation is the formulation developed by the joint partnership of MonoSol Rx and Midatech [47]. The two companies have combined their specialties, Midatech's gold nanoparticle (GNP) technology and MonoSol's PharmFilm drug delivery technology, to form a buccal soluble film product called MidaForm® Insulin PharmFilm [52,53]. In this formulation, recombinant human insulin is bound to glycan-coated GNP through noncovalent binding and embedded in a polymeric mucoadhesive film for the delivery of insulin via the buccal mucosa. It is claimed by Midatech Pharm that the GNP technology helps drugs to cross membranes, leading to an increase in stability. The particles are also inert and biocompatible [54]. Gold metal atoms form the core of the GNPs and these are attached via gold–sulfur bonds to an organic layer of glycans. During the self-formation process, insulin can attach to the gold core, which is 1–2 nm in size. In Phase I clinical trials, using insulin aspart, the formulation was shown to be both well tolerated and safe [47]. Additionally, owing to their small size, GNPs are believed to be eliminated via the liver and kidneys. Although the technology appears promising, and the company has facilities to scale up the production of the formulation, Midatech made an announcement in May 2016 that the results of the Phase IIa clinical trial (MTD101) demonstrated low bioavailability for the transbuccal film insulin compared to s.c. insulin and, hence, the company was evaluating its options [55]. The product is not on the pipeline list of products for either Midatech or MonoSol Rx, now renamed as Aquestive. Thus, it

TABLE 2

Clinical trials appearing on clinicalTrials.gov involving pulmonary, buccal, oral, and nasal insulin delivery systems, for T1DM and T2DM, with a trial start date being between January 2008 and December 2017[a]

Product Name	Company	Technology	Year trial started	Phase	NCT number	Refs
Pulmonary insulin delivery						
Dance-501	Dance Biopharm	Inhaled insulin administered using Adagio-01 inhaler device (also known as dance-501); device uses vibrating mesh micropump technology developed by Aerogen	2013	I and II	NCT02713841	[50]
			2016	I and II	NCT02716610	
Afrezza®	Mannkind and Sanofi	Technosphere® Insulin SAR439065 Afrezza®. Technosphere® particles formed using the carrier fumaryl diketopiperazine (FDKP). Insulin is adsorbed onto particles (~2 μm in size) forming Technosphere® insulin	2008	I	NCT00673621	[5]
				II	NCT00662857	
				I	NCT00674050	
				II	NCT00747006	
				III	NCT00700622	
			2009	III	NCT00642616	
			2010	III	NCT01196104	
			2011	III	NCT01451398	
				III	NCT01445951	
			2012	I	NCT01544881	
			2015	I	NCT02485327	
				I	NCT02470637	
				II	NCT02527265	
			2017	I and II	NCT03234491	
				III	NCT03324776	
				IV	NCT03143816	
				IV	NCT03313960	
Buccal insulin delivery						
Oral-lyn™	Generex Biotechnology	Formulation contains surfactants as absorption enhancers by forming insulin-containing micelles. Generex Oral-lyn™ delivers insulin via device known as RapidMist™	2008	III	NCT00668850	[45,47,48]
Oral insulin delivery						
GIPET® I	Novo Nordisk	GIPET® I tablet preparation comprises micelles formed with aid of patented absorption enhancers	2013	I	NCT01809184	[57]
			2013	I	NCT01796366	
				I	NCT01931137	
			2014	I	NCT02304627	
			2015	II	NCT02470039	
				I	NCT02479022	
ORMD-0801	Oramed and Integrium	ORMD-0801 comprises enteric-coated capsule that encompasses insulin along with protease inhibitors and absorption enhancers that aid delivery in small intestine	2008	II	NCT00867594	[59]
			2013	II	NCT01889667	
			2014	II	NCT02094534	
			2015	II	NCT02535715	
				II	NCT02496000	
			2016	II	NCT02954601	
Oshadi Oral Insulin	Oshadi Drug Administration	Oshadi carrier contains mixture of pharmacologically inert silica NPs with hydrophobic surface and branched polysaccharide. Insulin is suspended, embedded or dispersed in oil or mixture of oils	2010	I	NCT01120912	[64]
		Oshadi Icp (insulin, proinsulin, and C-peptide in Oshadi carrier)	2013	I and II	NCT01772251	
				II	NCT01973920	
HDV Insulin	Diasome Pharmaceuticals and Integrium	In hepatocyte-directed vesicle (HDV) insulin gel capsules, insulin is bound to HDV, which is <150 nm in diameter; phospholipid bilayer has specific hepatocyte-targeting molecules (HTM), which in the latest preparation is biotin-PE	2008	II and III	NCT00814294	[61]
			2016	II	NCT02794155	

TABLE 2 (*Continued*)

Product Name	Company	Technology	Year trial started	Phase	NCT number	Refs
IN-105	Biocon	Tablet formulation IN-105 oral insulin (now known as Tregopil) is modified form of human insulin in which free amino acid group on Lys-β29 residue is covalently bonded via a nonhydrolysable amide bond to small PEG molecule. This modification offers better stability and reduced degradation in presence of enzymes in gastrointestinal tract	2010	I	NCT01035801	[66,67]
ORA2	Bows Pharmaceuticals AG	Insulin in dextran matrix capsule	2009 2010	I and II I	NCT00990444 NCT01114750	
Nasal insulin delivery						
Nasulin™	CPEX Pharmaceuticals	Intranasal insulin spray contains CPE-215, which is main excipient in permeation enhancement technology of CPEX	2009	II II	NCT00850161 NCT00850096	[72,73]

[a] No trials recorded for transdermal delivery in the past decade [7].

appears unlikely that the development of MidaForm® Insulin PharmFilm will be advanced further.

Oral insulin delivery

Novo Nordisk in partnership with Merrion Pharmaceuticals are developing a gastrointestinal permeation enhancement technology (GIPET) system for the oral delivery of insulin in the form of a tablet [56]. The oral preparation comprises micelles formed with the aid of patented absorption enhancers, which increase absorption across the GI tract. In addition to having good reproducibility, as stated by the CEO of Merrion Pharmaceuticals, because of the use of generally recognised as safe (GRAS) ingredients, the GIPET formulation will also result in the development of oral products that are low risk and that have good safety profiles. The main product is known as GIPET I (OI338GT or NN1953), a long-acting insulin analogue, which has reached Phase II clinical trials. Novo Nordisk are also using the GIPET technology to develop oral formulations of two other insulin analogues, insulin 287 and insulin 320 (OI320GT or NN1957), which have both completed Phase I clinical trials [57].

Oramed Pharmaceuticals have an oral insulin formulation, known as the ORMD-0801 capsule, which is being tested in several Phase II clinical trials in patients with either T1DM or T2DM [58]. Oramed Pharmaceuticals' Protein Oral Delivery™ (POD) technology comprises an enteric-coated capsule that encompasses insulin along with protease inhibitors and absorption enhancers that aid delivery in the small intestine. In the Phase II trial (NCT00867594), eight patients with T1DM and uncontrolled blood glucose levels (HbA_{1c} 7.5–10%), were trialled with the ORMD-0801 capsules containing 8 mg insulin in each capsule [59]. In this trial, the

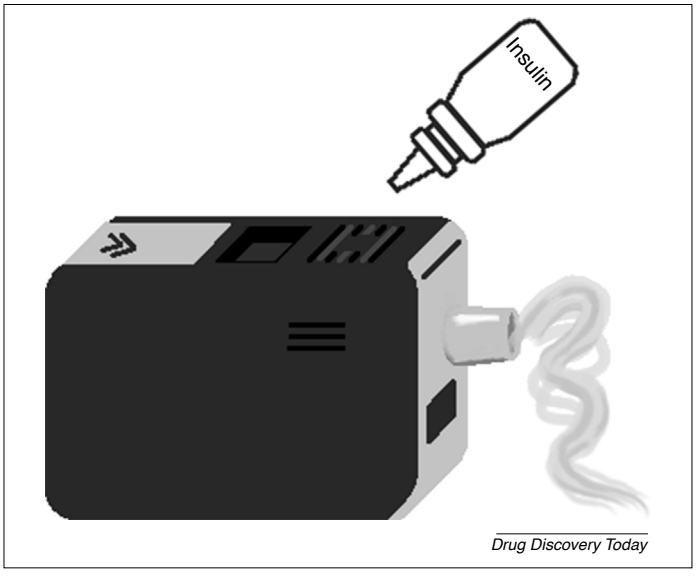

FIGURE 4

Simplified image of the Dance-501 device along with a separate insulin container. This device uses a vibrating mesh micropump technology developed by Aerogen. Adapted from Ref. [50].

patients were asked to self-administer the ORMD-0801 capsules three times a day, 45 min before their meals, in addition to their standard insulin therapy. Although the study included a small number of individuals, and a short treatment period of 10 days, the outcome was significantly reduced glycaemia throughout the day. Additionally, the formulation was tolerated well by the individuals, with no hypoglycaemic episodes or adverse events. A potential drawback to this formulation is the administration of a large amount of insulin and it is surprising that no hypoglycaemic episodes occurred within the study; however, the study was small and the effects of interindividual variability will be more likely to occur in larger studies.

Diasome Pharmaceuticals have designed an oral hepatocyte-directed vesicle (HDV) insulin gel capsule, in which all the insulin is bound to HDV. The HDV vesicle is <150 nm in diameter and the phospholipid bilayer has specific hepatocyte-targeting molecules (HTM), which, in the latest preparation, was biotin-phosphatidylethanolamine (biotin-PE), incorporated within its structure [60]. As reviewed by Geho *et al*. [60], this formulation not only improved oral insulin delivery by shielding insulin from proteolytic enzymes in the upper GI tract, but additionally was more able to mimic physiological insulin delivery via HTM guidance towards hepatocytes. Thus, this novel carrier system appears promising and has been approved by the FDA for initiation of Phase II clinical trials based on the effectiveness of the delivery system in Phase II studies in humans. Compared with other possible oral insulin formulations, one of the most promising aspects of this formulation is the amount of insulin contained in each capsule, which can be as little as 5 U [43,61]. Accuracy of dosing is important with insulin treatment and one of the main disadvantages with insulin being given orally is the possibility that interindividual variability can lead to overdosing, resulting from not only genetic variations in individuals, but also factors such as food and transit time in the GI tract; in theory, the risk would be lower with formulations containing smaller amounts of insulin, such as with HDV oral insulin compared with formulations with 150 U or more per dose, such as Capsulin™ IR, designed by Diabetology [62].

Oshadi Drug Administrations' newest formulation is in Phase II clinical trials and is called Oshadi Icp, because it is a combination of insulin, proinsulin, and C-peptide. In 2010, the company made a patent application for their oral insulin formulation in which it is stated that the preparation comprised 'a particulate non-covalently associated mixture of pharmacologically inert silica nanoparticles having a hydrophobic surface, a branched polysaccharide, and insulin suspended, embedded or dispersed in an oil or mixture of oils' [63]. The diameter of the NPs is in the range of 1–100 nm. Other than the information obtained from the patent (20100278922), little other information has been published. The possible reasoning for the combination of insulin, proinsulin, and C-peptide in the formulation is to reflect a delivery system similar to endogenous insulin release, particularly because orally delivered insulin reaches the circulation via the hepatic portal vein [64].

Biocon's oral insulin tablet IN-105, now known as Tregopil, is a novel insulin analogue [65]. It is a modified form of human insulin where the free amino acid group on the Lys-β29 residue has been covalently bonded via a nonhydrolysable amide bond to a small polyethylene glycol (PEG) molecule [66]. This modification, compared with the original human insulin, has the advantage of better stability and reduced degradation in the presence of enzymes in the GI tract possibly because of steric hindrance. The water solubility of the insulin analogue is also improved, probably because of the presence of the PEG modification. Furthermore, in Phase I studies, it was found that the amount of insulin absorbed was adequate enough to reduce plasma glucose levels, which indicated that the alteration aided absorption of the intact insulin peptide in the GI tract. After administration, based on the initial studies, it was observed that insulin levels peaked at around 20 min and returned to baseline after 1 h 20 min, suggesting that Tregopil could be useful to control postprandial glucose levels [66].

Diabetology (not included in Table 2) is another company that has developed an oral insulin product, known as Capsulin™ IR (insulin replacement), which is in Phase II clinical trials [67]. The formation contains unmodified insulin (150 U or 300 U) and is a simple mixture contained in a standard enteric-coated capsule [62]. The oral formulation uses the company's in-licensed Axcess™ drug delivery technology, which contains a solubiliser and absorption enhancer, but no new chemical entities (NCEs), and has demonstrated effectiveness in delivering peptides, such as insulin [68]. The main excipients are both pharmacopoeial and GRAS listed and include an aromatic alcohol and dissolution aid. The formulation has been designed to bypass the harsh pH conditions of the stomach and to rapidly dissolve in the small intestine (jejunum), allowing all the components to come into contact with the surface of the intestinal wall.

Bows Pharmaceutical AG were also developing an oral insulin formulation comprising insulin in a dextran matrix capsule. However, it appears that they are no longer active in developing the product, with the last update being from November 2010 [7].

Given that insulin is hydrophilic, the main route of permeation in the GI tract is via the paracellular route [69]. Being a protein, the permeability of insulin as well as its oral bioavailability, is likely to be low without any absorption enhancement [70]. Therefore, most of the oral insulin formulations being developed include absorption enhancers, although the likely toxicity of such products is a concern in the long term.

Nasal insulin delivery

CPEX Pharmaceuticals Inc. has developed an intranasal insulin spray, containing regular short-acting human recombinant insulin, and given the trade name Nasulin™. The main excipient in the nasal insulin formulation is cyclopentadecalactone (CPE-215), which, according to the company, has been proven to enhance absorption [71,72]. CPE-215 is a naturally occurring compound obtained from the plant *Angelica archangelica* and is contained in many everyday-use products, including food ingredients, cosmetics, and personal hygiene products. The other components of the formulation include polysorbate 20, sorbitan monolaurate, and cottonseed oil. During initial studies in healthy volunteers, it was found that the normal physiological nasal cycle did not result in clinically significant alterations in insulin absorption, although the absorption of insulin was decreased by ~50% in those individuals who were affected by total nostril blockage [73]. Insulin levels peak at around 10–20 min post administration and, hence, the formulation is suitable for prandial glucose control. Nasulin™ is generally well tolerated, although transient adverse effects, such as

irritation, tickling sensations, and sneezing, do occur but tend to disappear on continued dosing. In the study by Stote *et al.* [72], it was concluded that, overall, the intrasubject variability in insulin administered using Nasulin™ was ~40%, comparable to that of normal s.c. administration.

Transdermal insulin delivery

Although no transdermal formulations have been reported over the past decade in the ClinicalTrials.gov database, recent searches of reviews show a possible transdermal formulation that is in the later stages of clinical trials. It is the non-invasive Ultrasonic Strip (U-Strip) transdermal patch designed by Transdermal Specialities, which can be used by patients with either T1DM or T2DM [74]. This is a two-component system, comprising an insulin patch, which uses an absorbent pad containing up to 150 U of insulin, attached to the second component, the U-Strip controller, which is a transducer device that generates a unique alternating ultrasonic transmission [75]. The U-Strip system utilises two types of ultrasonic waveform: initially, sawtooth waveforms are used to expand the pore diameter from ~50 μm to 110 μm to facilitate the penetration of large molecules, such as insulin; then, square waveforms are emitted to actively force insulin through the enlarged pores into the dermis and into the blood circulation. The U-Strip device is portable, battery operated, and designed to release insulin, specifically insulin lispro, only when the ultrasound is activated. It is also programmable, can be worn on either one of the arms or on a belt, and has a touch screen to enable patients to control both dosage levels and frequency. Additionally, the device can store data, which are transferable via the internet and, hence, can be a useful record for the management of the patient's treatment and compliance by their healthcare team. This information was provided by Bruce K. Redding, Jr, who is the president of Transdermal Specialties. Given that 12 human clinical trials have been completed successfully and an additional 500-patient trial is underway, the technology looks promising and it will be interesting to see further developments in the future.

Overview of recent therapies

Afrezza® is a newer, non-invasive, rapid-acting inhaled human insulin product, produced by MannKind, that is currently in clinical use in the USA [49]. It was approved for adults with either T1DM or T2DM by the FDA in June 2014, although in the UK and Europe, it is currently in Phase III clinical trials [5,76]. The safety and efficacy of Afrezza® was assessed in 3017 patients before the formulation was approved by the FDA. The insulin for this portable inhaler product is a dry powder formulation, which is contained in single-use cartridges comprising 4, 8, or 12 U doses of insulin [77]. Upon inhalation, which is powered by the patient's force of inhalation, the Technosphere® insulin particles are aerosolised and delivered to the lung alveoli [78]. Technosphere® particles are formed using the carrier fumaryl diketopiperazine (FDKP), which, under acidic conditions, self-assembles into microparticles, and this allows for the recombinant human insulin to adsorb onto the particles (~2 μm in size) and form Technosphere® insulin [5,79]. The formed microparticles have median diameters of ~2–2.5 μm, which is a suitable particle size for the delivery of molecules to the deep lungs. The alveolar fluid of the deep lungs provides an ideal environment for the rapid dissolution of Technosphere® insulin; because of the high solubility of the FDPK molecule in water at neutral or basic pH. After absorption across the alveoli into the bloodstream, the FDKP molecule remains unchanged and is excreted by the kidneys [78]. Although Afrezza® can be substituted for mealtime insulin, patients will still need to inject their long-acting basal insulin [80]. The pharmacokinetic and pharmacodynamic properties of Afrezza® was assessed in 30 patients with T1DM in a recent randomised, controlled six-way, crossover dose–response study [79]. The study looked at the comparison of inhaling 4, 12, or 48 U of insulin using Afrezza® compared with s.c. administration of 8, 30, or 90 U of insulin lispro. For inhaled insulin, the onset of action was around 12 min, the peak effect occurred between 35 and 55 min and baseline levels were reached around 90–270 min after administration. By contrast, the peak action for insulin lispro was around 90–180 min with baseline levels reached between 360 to 660 min. Nonetheless, the baseline levels for both Afrezza® and insulin lispro were noted to have been reached in a dose-dependent manner. Based on the data in this study for insulin lispro, as well as the results for Humalog® published on the electronic medicines compendium and shown in Table 3, it can be seen that, compared with s.c. administration, inhaled Afrezza® is both faster in onset and has a shorter duration of action [81]. As shown in Fig. 3, although there are many advantages for delivering insulin via the pulmonary route, there are also several disadvantages, some of which are experienced with Afrezza®, and it is not suitable for patients with chronic lung disease because of the risk of acute bronchospasm, and is not recommended for smokers or those who have recently quit smoking [49].

Another product of interest is the Generex Oral-lyn™ (or Oralin™) spray, produced by the Canadian company Generex Biotechnology, for use as prandial insulin in patients with either T1DM or T2DM. It has been approved for clinical use in Ecuador and Lebanon and, in Canada, USA, and Europe, the product is still in Phase III clinical trials [6,43,82]. Oral-lyn™ was approved by the FDA for treatment of patients under the Investigational New Drug (IND) program, which allows the drug to be accessible to patients with serious or life-threatening T1DM or T2DM who are not eligible for Phase III clinical trials and for whom there are no other alternative satisfactory treatments [82]. The insulin within the preparation is stable at room temperature for at least 6 months and is formulated using a combination of absorption enhancers and small amounts of excipients classified by the FDA as GRAS [43,83]. In the formulation, surfactants, which form insulin-containing micelles, are used as absorption enhancers and this appears to be a crucial component of the Generex drug delivery system. Generex Oral-lyn™ delivers insulin via a device known as RapidMist™, which is similar in appearance to metered dose systems used in asthma and chronic obstructive pulmonary disease treatment [84]. It delivers regular human insulin to the buccal mucosa; the insulin-containing micelles are >7 μm and, hence, are too big to reach alveoli and are impacted in the buccal cavity. Each canister contains 400 U of insulin and, although one puff holds 10 U of insulin, only 10% of the drug is absorbed. Thus, with each spray, 1 U of insulin is delivered to the blood circulation [43,84]. In practice, many patients require 10 U or more of insulin with each

TABLE 3

Comparison of the pharmacokinetic and pharmacodynamic profiles of a current s.c. rapid-acting insulin and the newer buccal and inhaled insulin formulations

Product	Type of insulin	Route and/or method of administration	Onset of action (min)	Peak action (min)	Time for effect to return to baseline (min)	Refs
Humalog®	Rapid-acting insulin lispro	Injected into s.c. tissue	~15	~90	~120–300	[81]
Oral-lyn™	Regular human insulin	Sprayed on buccal membrane	~32	~44	~85	[86]
Afrezza®	Recombinant human insulin	Inhaled for absorption in deep lungs	~12	~35–55	~90–270	[79]

meal, some considerably more, and so using the device to administer ten or more puffs each time could be become inconvenient and might not be feasible in the long-term.

In a comparative study of insulin levels in patients with T1DM, where Oral-lyn™ insulin was compared to s.c. injections of insulin or placebo, it was observed that, with Oral-lyn™, the entry of insulin into the blood circulation was faster compared with s.c. injections and insulin levels could be detected within 10 min of administration [85]. Additionally, Oral-lyn™ insulin reached peak insulin levels at 30 min, whereas s.c. injections were much slower. At 150 min, Oral-lyn™ insulin had almost reached baseline levels, whereas s.c. injections of insulin were still around peak levels. This small study, together with another study (data shown in Table 3), suggests that buccal insulin is more reflective of a normal insulin response to meal intake in individuals without DM compared with s.c. injections, taking less time to reach peak activity and having a shorter duration of action [85,86]. Although there are some advantages of the Oral-lyn™ device and formulation, its low bioavailability could be a major drawback to the product obtaining approval worldwide.

Concluding remarks and future directions

Insulin forms a major part of the diabetes treatment plan. In the future,the ideal and most convenient situation for patients with this disease would be to take insulin orally in the form of a tablet or capsule, although, based on the challenges of delivering proteins via the GI tract, the success of such a concept needs further development. On a positive note, even though many have failed, most trials in relation to insulin are related to oral delivery products. Hence, there is the possibility that an orally delivered insulin formulation will be approved by regulatory authorities (e.g., EMA and FDA) in the next decade or so. The challenge is finding a formulation that is clinically effective, safe,and convenient for long-term daily administration. If this is achieved, it will likely be for a long-acting basal insulin formulation to minimise the use of excipients, such as absorption enhancers or enzyme inhibitors, and also to overcome challenges, such as the fed and fasted state, and to avoid the crucial timing associated with reducing meal time glucose levels in the systemic circulation. One of the main products that appear promising is the oral HDV insulin gel capsule, because this formulation allows for a low dose of insulin to be used and, as such, has the theoretical benefit of having a lower risk of hypoglycaemia compared with other oral preparations that contain higher doses of insulin.

Two other promising non-invasive products are the Dance-501 handheld insulin inhaler device and the U-strip transdermal insulin patch system. The Dance-501 device has overcome many of the weaknesses faced by Exubera and has several advantages in terms of its design: it is small, discrete, and more patient friendly for use outside the home. However, this new inhaled insulin delivery system has the drawback of requiring the administration of ten times more insulin to obtain the equivalent amount of insulin from s.c. administration. If the bioavailability can be improved, the Dance-501 device could have a promising future. The U-Strip system is unique because the transdermal delivery of insulin is enhanced by combining two types of ultrasonic waveform. Although a relatively bulky controller is currently required, the technology appears promising overall and the outcome of current clinical trials are eagerly awaited. Although Afrezza® has been FDA approved, it still faces several challenges, including obtaining approval in Europe. The Generex Oral-lyn™ spray also requires approval in Europe, the USA, and many other countries worldwide. Once these two products become approved and in common use in Europe and the USA, then non-invasive insulin administration will become a reality and allow for newer formulations to have a better opportunity of success and approval. There are many challenges to overcome but developing a formulation that can replace s.c. injections would not only be a great accomplishment scientifically, in terms of delivering a protein safely for chronic use non-invasively, but also a major contribution to easing the lives of millions of patients and reducing healthcare costs worldwide.

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