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Intestinal toxicological and inflammatory impact of oral delivery of macromolecules: focus on Diabetes

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ÍNDICE

Abstract

Nanotechnology-based approaches towards an oral delivery of macromolecules such as insulin are increasingly therapeutic strategies for the prevention or treatment of diabetes. However, the intestinal absorption of insulin may be associated with an immunostimulatory reaction induced by the complement system. Intestinal epithelium is an immune privileged organ, it able to mediate immune reactions either playing a local protective role or by triggering an inflammatory response to the presence of exogenous molecules, pathogens or nanoparticles (NP).

Compromising safety of insulin delivered by nanoparticles, by inadequacy or insufficiency of studies, may lead to exacerbation of the inflammatory pathways conducive to unwanted local and other severe adverse effects. Therefore, it is imperative to include wide ranging immunological and toxicological assays in early pre-clinical studies in order to increase the representativeness of the results and strengthen the potential for progression into further stages of research.

Herein, focus will be put on recent reports in oral delivery of insulin nanoparticles for Diabetes as well as the suitability of the safety studies supporting their pre-clinical development. The improvement of early safety assessment by transitioning to quantitative, structure-immune performance relationship studies in representative models will also be elucidated. Thereby, the role and importance of rational optimization in the development of "safe by design" nanotechnology will be contextualized in the field of Diabetes.

Keywords: Diabetes; Intestinal epithelium; Nanoencapsulation; Oral insulin; Safety

Resumo

A administração oral de macromoléculas como a insulina recorrendo a nanotecnologias é uma estratégia terapêutica cada vez mais importante para a prevenção ou tratamento de diabetes. Contudo, a absorção intestinal de insulina pode estar associada a uma reacção imunoestimulante induzida pelo sistema do complemento. Considerado como um órgão imunitário privilegiado, o epitélio intestinal é capaz de mediar reacções imunitárias através de um papel protector local ou de desencadeador de uma resposta inflamatória à presença de moléculas exógenas, agentes patogénicos ou nanopartículas.

A falta de segurança por inadequação ou insuficiência de estudos pode levar à exacerbação das vias inflamatórias conduzindo a efeitos adversos graves locais. É, portanto, imperativo incluir em estudos pré-clínicos uma ampla gama de ensaios imunológicos e toxicológicos de modo aumentar a representatividade dos resultados e reforçar o potencial para a progressão em outras fases do desenvolvimento de nanopartículas para da administração oral de insulina .

Nesta revisão, são focados estudos recentes da administração oral de insulina para a diabetes, bem como a adequação dos estudos de segurança que suportam o seu desenvolvimento pré-clínico. O melhoramento dos processos de avaliação precoce de segurança através de uma transição para estudos quantitativos de relacionamento de desempenho estrutura-imune em modelos animais representativos será também elucidado. Assim, o papel e a importância da optimização racional no desenvolvimento de nanomedicamentos "safe by design" será contextualizada no campo da Diabetes.

Palavras-chave: Diabetes; Epitélio intestinal; Insulina oral; Nanoencapsulação; Segurança

1 Introduction

Diabetes *mellitus* (DM) is a chronic, progressive, medically incurable disease and is poorly controlled in a vast majority, in spite of tremendous advancements in pharmacotherapy. (Sanyal, 2013)

It is estimated that the worldwide prevalence of diabetes in adults (20 to 79 yr of age) was 6.6% (285 million people) in 2010 and will increase to 7.8% (438 million people) by the year 2030. (Card & Magnuson, 2011) Today, accordingly with the World Health Organization site (accessed on July, 12) 347 million people have diabetes. Intensive insulin in Type 1 Diabetes (T1D) patients is able to reduce the risks of nephropathy by 35% to 56%, neuropathy by 60%, and retinopathy by 50% to 70%. (WHO, 2014; Fonte et al, 2013)

Subcutaneous (SC) insulin therapy is a common mode of diabetes treatment, which significantly reduces morbidity and mortality, but can be burdened by complications (hypoglycemia, edema) and low patient compliance owing to the use of needles and the complexity of the insulin treatment regimen, the late stage at which insulin may be prescribed, and fear of hypoglycemia episodes and weight gain. To overcame such hurdles considerable effort has been put on the oral development of oral insulin formulations that can provide bioactive insulin in a non-invasive manner posing minimal patient risk. The oral route remains the preferred choice for drug administration because of its non-invasive nature. However, proteins such as insulin have low oral bioavailability due to their intrinsic lack of permeability through the intestinal epithelium. The development of a delivery system intended to provide insulin orally, requires a proper understanding about the mucosal microenvironment and intestinal physiology. Such efforts are important as the identification of factors that influence the immune responses underlying the pathogenesis of T1D could provide an opportunity for therapeutic measures to prevent and/or treat the disease, as well as allow for the development of improved biomarkers for predicating future cases of the disorder and other chronic diseases as well.

Proteins and peptides are the building blocks of life and are now evolving as a very promising brand of therapeutic entities. (Muheem et al, 2014) Accumulating data indicate that dysregulation of the gut immune system may play a fundamental role in the development of beta cell autoimmunity and type 1 diabetes. (Vaarala, 2002) The gut immune system has a dual nature: exposure to oral antigens may lead to tolerance and/or immunization.

The term ‘gut microbiota’ represents a complex microbial community within the body, one capable of affecting health by contributing to nutrition, prevention of colonisation

of the host by pathogens, and through influencing the development and maintenance of the immune system. Experimental evidence suggests that alterations in the gut microbiota are associated with the development of a number of disorders attributed to an overly activated immune system/autoimmunity (e.g. ulcerative colitis, Crohn's disease), including an influence on type 1 diabetes. (Atkinson & Chervonsky, 2012)

Potential protein therapies that have failed so far outnumber the successes, mainly due to a number of challenges that are faced in the development and use of protein therapeutics, among which route of administration is a critical factor in any therapeutic intervention that governs both the pharmacokinetics and efficacy of the protein. (Muheem et al, 2014) The United States Food and Drug Administration (FDA) laid down the foundation for the popularity of protein therapeutics with the regulatory approval of recombinant insulin in 1982.

The following review will focus on recent reports in oral delivery of insulin for Diabetes as well as the suitability of the safety studies supporting their pre-clinical development. The improvement of early safety assessment by transitioning to quantitative, structure-immune performance relationship studies in representative models will also be elucidated. Thereby, the role and importance of rational optimization in the development of "safe by design" nanotechnology will be contextualized in the field of Diabetes.

2 Peroral administration: the promising route

The route of administration is a critical factor in any therapeutic intervention that governs both the pharmacokinetics and efficacy of insulin. Its high molecular weight and hydrophilicity hinder their intestinal absorption, leading to low oral bioavailability, negligible plasma levels, and high variability. Various challenges, such as overcoming enzymatic degradation, solving the problem of poor absorption in the gastrointestinal tract (GIT) and preserving the insulin's biological activity during the formulation process have to be overcome. (Muheem et al, 2014; Ruiz et al, Sep 2014) This has prompted researchers to develop new delivery systems capable of delivering insulin in a more effective manner.

Peroral route continues to be the most intensively investigated alternative route for insulin delivered by parenteral route. This interest in the peroral route, despite enormous barriers to drug delivery that exist in the GIT, can be very well appreciated from obvious advantages such as ease of administration and large patient acceptability. Potential cost savings to the health care industry further augment the advantages of peroral systems in

terms of patient compliance and acceptability, since peroral formulations do not require sophisticated sterile manufacturing facilities or the direct involvement of health care professionals.

Although the oral delivery of insulin remains an attractive option, to reach its true potential the challenges must be met. Oral delivery of proteins and peptides has long been hailed as the 'Holy Grail' of drug delivery by showing great potential but also presenting problems in their development. (Muheem et al, 2014)

The main barriers to the intestinal absorption of insulin and a clinically usable oral insulin formulation are the low permeability of proteins across the intestinal wall coupled with high susceptibility to acid denaturation in the stomach and enzymatic degradation throughout the gut.

2.1 Oral insulin delivery systems

Insulin is released from pancreatic beta-cells into the hepatic portal vein and then into the liver, which is the primary site of action, whereas parenteral route and other delivery systems (buccal, pulmonary, and nasal) deliver the drug directly into the systemic circulation. In this delivery system, the drug reaches the systemic circulation bypassing the first-pass metabolism, but in case of oral delivery, insulin first reaches the liver (20% of drug dose is available in liver) and then to the peripheral tissue. Thus, oral route of administration is closer to the natural physiological route of insulin. (Muheem et al, 2014)

An ideal delivery system for oral administration of insulin should prolong its intestinal residence time, reversibly increase the permeability of the mucosal epithelium to enhance the absorption of drug and provide the intact drug to the systemic circulation. Additionally, this delivery system must be safe after oral administration. (Sonaje et al, 2009)

Nanoparticles are able to permeate the intestine by different pathways (Figure 3). In the case of insulin, it is adsorbed on the apical membrane and is internalized by specific types of endocytosis processes.

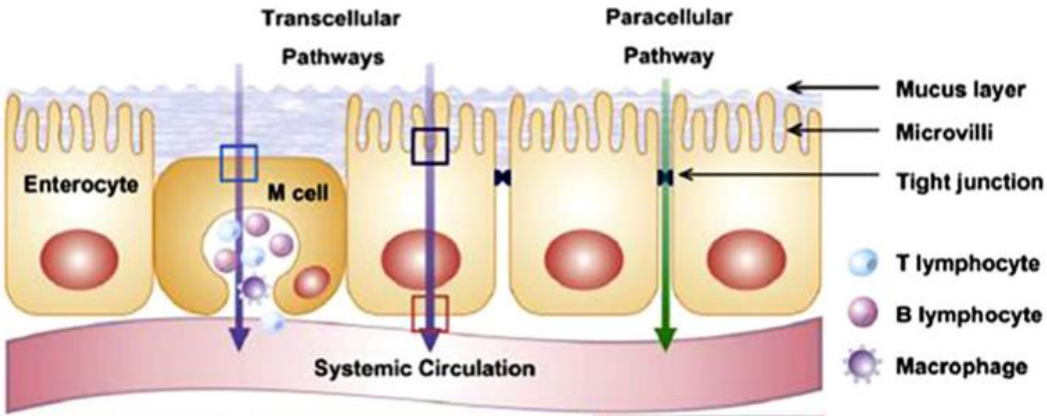

Figure 3 – Pathways for insulin nanoparticle translocation through the intestinal epithelium. (Fonte et al, 2013) © 2014 Elsevier B.V.

3 Diabetes and Intestinal Immune System

Diabetes is a chronic disease, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces (WHO, 2014).

Type 1 diabetes (T1D) is characterized by a lack in insulin production while Type 2 diabetes (T2D) is caused by the body's ineffective use of insulin.

The link between the gut immune system and T1D has been suggested by studies showing dietary factors influence on the disease in animal models of autoimmune diabetes. (Vaarala et al, Oct 2008) T1D is a disease that may involve autoimmune destruction of pancreatic beta cells in genetically predisposed individuals and evidence suggests a particular role for intestinal microbiome alterations in autoimmune disease development, including T1D. (Boerner & Sarvetnick, 2011)

The human intestinal microbiome is a symbiotic ecological community that influences human health and development, including the development maintenance of the human immune system. The intestinal mucosa is a common entry site for pathogens and harbors a significant proportion of the cells of the immune system. An intact mucosa provides the first line of defence against pathogens and exogenous matter. (Atkinson & Chervonsky, 2012)

With the importance of the gut microbiota for autoimmunity established, the mechanisms by which exogenous matter such as nanoparticles, specifically influence the pathogenesis of Diabetes has became the focus of immunological research.

3.1 Intestinal immune system

The intestine forms a barrier not only against invading agents but also against dietary antigens and commensal bacteria. The mucosal barrier (Figure 1) consists of an extrinsic barrier with non-specific defence mechanisms, i.e., low pH, digestive enzymes, mucus, and peristalsis, and an immunological barrier with secretory IgA (immunoglobulin A) and IgM (immunoglobulin M), whereas the intrinsic barrier is based on the integrity of the intestine itself. (Paronen, 2001)

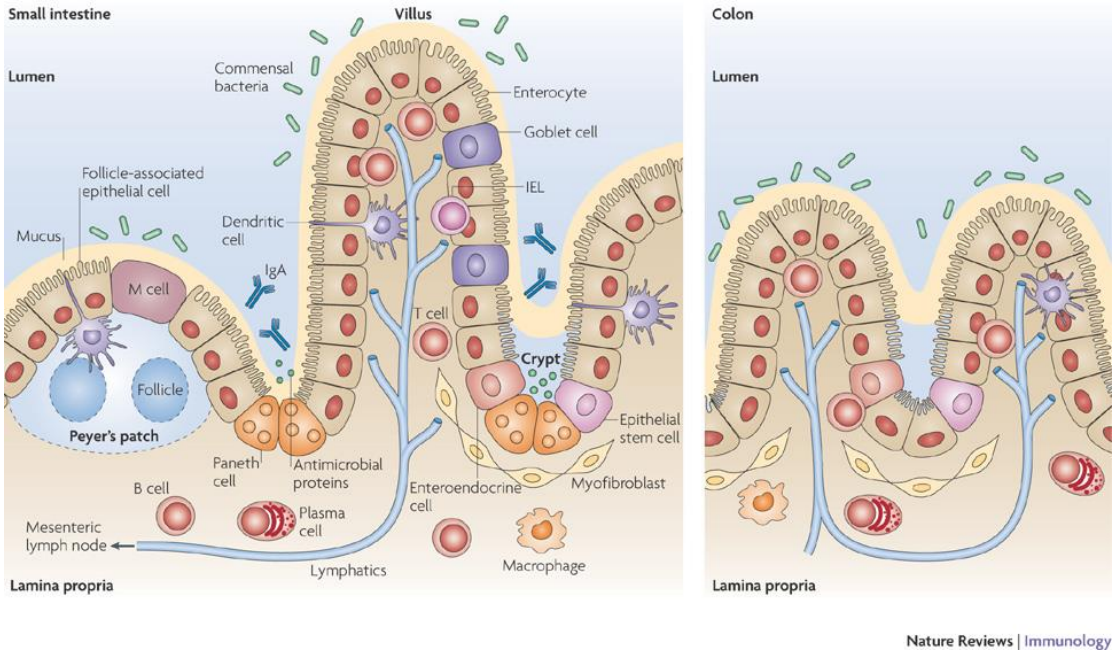

Figure 1 – Anatomy of intestinal immune system. A single layer of intestinal epithelial cells (IECs) provides a physical barrier that separates the trillions of commensal bacteria in the intestinal lumen from the underlying lamina propria. © 2014 Macmillan Publishers Limited.

The complexity of the immune system reflects the multiple, complementary and sometimes opposing functions that are necessary to maintain a healthy organism. These functions range from defence against invasive pathogens to capacity of tolerating innocuous substances to which the intestine is exposed and self-components of the body.

Mucosal surfaces, such as the intestine, represent a barrier between the human body and the external environment. (Sorini & Falcone, 2013) (Figure 2) Specialized epithelial cells constitute barrier surfaces that separate mammalian hosts from the external environment. The GIT is the largest of these barriers and is specially adapted to colonization by commensal bacteria that aid in digestion and markedly influence the development and

function of the mucosal immune system. However, microbial colonization carries with it the risk of infection and inflammation if epithelial or immune cell homeostasis is disrupted. The capacity to maintain the segregation between host and microorganism is essential to keep coexistence of commensal microbial communities and mucosal immune cells. The intestinal epithelium accomplishes this by forming a physical and biochemical barrier to commensal and pathogenic microorganisms. Intestinal epithelial cells (IECs) can sense and respond to microbial stimuli to reinforce their barrier function and to participate in the coordination of appropriate immune responses, ranging from tolerance to anti-pathogen immunity. This way, IECs maintain a fundamental immunoregulatory function that influences the development and homeostasis of mucosal immune cells. (Peterson & Artis, 2014)

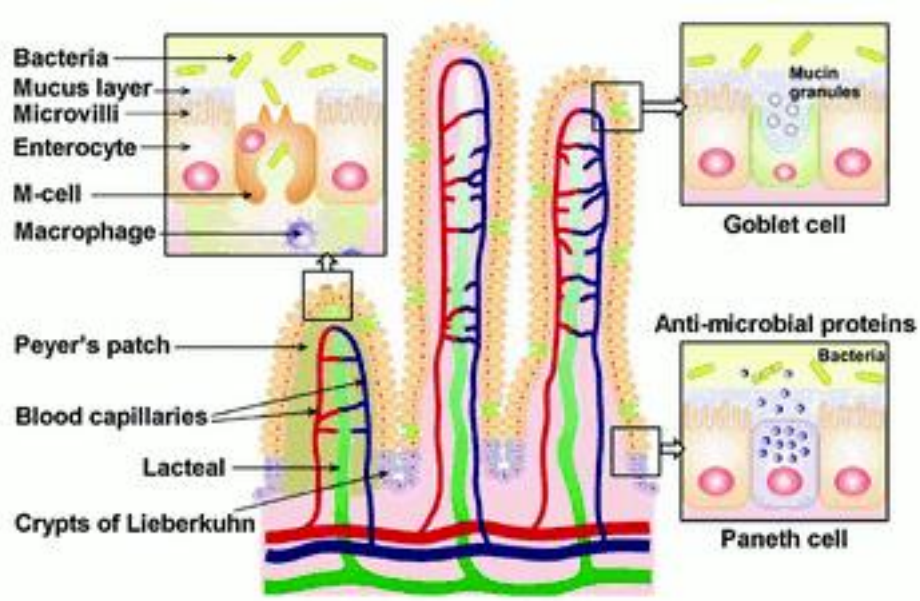

Figure 2 – Schematic illustrations of the intestinal epithelium (MO et al, 2014) © Royal Society of Chemistry 2014

Enterocytes (absorptive) and goblet cells (mucus secreting) cover the villi, which are interspersed with Follicle Associated Epithelium (FAE). These lymphoid regions, Peyer's patches, are covered with M cells specialized for antigen sampling. M cells are significant for drug delivery, since they are relatively less protected by mucus and have a high transcytotic capacity. (Ensign et al, 2012)

Foreign exogenous particles that enter the body throughout the intestinal mucosa must be rapidly eliminated by a protective immune response. By contrast, the intestine is normally home to a variety of commensal bacteria and is continuously exposed to large

quantities of food proteins. As a result, the intestinal immune system has to discriminate between invasive organisms and harmless antigens, inducing immunological tolerance towards the latter. The anatomical organization and peculiar cell subsets that are present in the gut mucosa reveal the complexity of its functions. (Sorini & Falcone, 2013)

The microbial colonization is influenced by many factors including mode of delivery, type of feeding, and the widely used antibiotic therapy. Since microbiota influences the immune system through their ability to affect immune responses to pathogens and commensals, it is likely that autoimmunity would (directly or indirectly) be affected as well.

Although the evidence for the role of the gut immune cells in the pathogenesis of beta-cell autoimmunity is indirect (Table 1), this possibility is fascinating since it may lead to the development of novel strategies for diabetes prevention. (Vaarala, 1999)

Table 1 – Evidence for the role of the gut immune system in the pathogenesis of Type 1 diabetes (Vaarala, 1999; Vaarala, 2002)

Animal models of autoimmune diabetes	Human Type 1 diabetes (T1D)
Diet modifies the incidence of diabetes in NOD-mice and in BB-rats	Cow milk exposure implies a risk of diabetes
Lymphocytes expressing mucosal adhesion molecules infiltrate the islets in NOD-mice	Enhanced immune responsiveness to food antigens occurs in the patients with T1D
Monoclonal antibodies to MadCAM-1 and b 7-integrin prevent autoimmune diabetes in NOD-mice and in adoptive transfer model	GAD-reactive T-cells express gut associated homing receptor
Diets with low diabetogenecity induce Th2 type cytokine profile in the islet-infiltrating T cells	Mucosal homing of lymphocytes derived from human diabetic pancreas
Mesenterial lymphocytes from a young NOD mice transfer diabetes to healthy recipients	Increased permeability of the gut
Feeding autoantigen may prevent or accelerate autoimmune diabetes	Association of celiac disease with type 1 diabetes
Dietary manipulations cause changes in the cytokine profile of the islets infiltrating lymphocytes in BB-rats	Immunohistology of intestine shows markers of inflammation in T1D

3.2 Humoral immune response to insulin

Insulin is one of the major autoantigens of T1D and has some unique features, compared with other autoantigens. Insulin is the major product of pancreatic islet beta-cells, which are the specific target of autoimmune destruction. Insulin is the only T1D-associated autoantigen that is exclusively expressed in the beta-cells, with the exception of self-antigen expressing cells in lymphoid tissues such as the thymus, where insulin is expressed at low levels without hormonal importance. The other autoantigens are expressed in other islet cells and in other tissues in addition to the beta-cells. Insulin is secreted into the bloodstream and is a ubiquitous antigen in this sense. (Ruiz et al, Sep 2014)

In humans, insulin was the first autoantigen identified to which autoantibodies were proven to exist. (Ruiz et al, Sep 2014; Tiittanen, 2006)

Environment plays a crucial role in shaping the immune system in both physiologic and pathologic conditions and modulates the pathogenesis of autoimmune diseases such as T1D. The gut microbiota can have a fundamental role as intermediary between the high number of environmental triggers that alter autoimmune processes and the diverse immune cells, possibly including autoimmune T cells that patrol our mucosal surfaces. Furthermore, direct links between microbiota alterations and autoimmunity have been found. The discovery that specific nutrients and dietary supplements can selectively affect colonization capacity of either beneficial or detrimental species of the microbiota open to the possibility of their therapeutic exploitation in the prevention or treatment of autoimmune diseases. (Burcelin, 2012; Sorini & Falcone, 2013; Vaarala, 1999) All of these factors together with certain genetic factors may influence the regulation of immune responses in the gut immune system and possibly influence the development of beta-cell autoimmunity. Prevention strategies of T1D based on the manipulation of the gut immune system may provide new realism to the search for a cure to autoimmune diabetes. (Vaarala, 1999)

4 Toxicological and safety issues

Toxicity is a critical factor to be considered when evaluating the potential of insulin-loaded nanoparticles. Given that nanoparticles are designed to interact with cells, it is important to ensure that they do not cause any adverse effects or even damage the intestinal epithelium. The important issue is that, whether uncoated or coated, nanoparticles will undergo biodegradation in the cellular environment and may affect cellular responses. For

instance, biodegraded nanoparticles can accumulate inside the cells and lead to intracellular changes, such as disruption of organelle integrity or gene alterations, which cause severe toxicity. (Fonte et al, 2013)

Cytotoxicity may not be the only adverse effect because cell immunological response may also be affected. Furthermore, molecules delivered to unnatural sites in unnatural quantities are likely to behave in unexpected ways, so from a toxicological perspective, oral delivery of macromolecules such as insulin may be questionable. If insulin is entrapped and not released from carrier systems until it reaches the systemic circulation, then this may not be an issue, but this approach is questionable, because insulin may cause gastroparesis. (Fonte et al, 2013) The use of absorption enhancers may lead to a long-term toxicity, and surfactants can also damage intestinal epithelium as well. Indeed, absorption enhancers, when administered in a continuous manner, may also promote permeation of pathogens and toxins. Moreover, mucoadhesive systems may affect mucus turnover and consequently alter the physiology of intestinal membrane. (Fonte et al, 2013)

5 Current safety and toxicology studies

It is known that the natural pH environment in the GIT varies from acidic in the stomach to slightly alkaline in the small intestine. Studies reported that mucoadhesive properties of nanoparticles were found to be affected by the pH conditions in the small intestine also that with increasing pH the amount of insulin transported decreased significantly. (Sonaje et al, 2009) Results of *in vivo* toxicity studies demonstrated that there was no apparent toxicity observed for the animals treated with empty nanoparticles, even with a dose 18 times higher than that used in the pharmacokinetic study. (Sonaje et al, 2009)

Other studies reported no increase in hemolysis in the presence of the nanoparticles, suggestive of suitable blood compatibility epithelial integrity and cellular tight junctions in the ileum remained intact in the presence of the nanoparticles. No statistical differences from the control values were noted in any of the measured outcomes, and no evidence of gross or histological abnormalities was reported, suggesting that the nanoparticles were well tolerated over the course of a 14-day repeat-dose regimen. (Sonaje et al, 2009)

A large number of clinical trials were retrieved from the clinical trials registry of the U.S. National Library of Medicine (www.clinicaltrials.gov; accessed on July 5, 2014) when a search was conducted using the terms "oral AND insulin". Some studies about safety and

efficacy of administration of oral insulin are completed, other are waiting for recruitment and others were suspended (Table 2).

No published data is available yet and it is unclear how many of these pertain to nanoparticle-based delivery systems.

Product Name	Company	Technology	Status
Capsulin	Diabetology (Jersey, UK)	Axcess ™; enteric-coated capsule filled with a mixture of insulin, an absorption enhancer, and a solubilizer	Phase IIa in T1D and Phase II in T2D completed; agreement with USV Limited (Mumbai India) to complete the development and commercialize for Indian market
ORMD-0801	Oramed (Jerusalem, Israel)	Enteric-coated capsule containing insulin and adjuvants to protect the protein and promote its intestinal uptake	Phase IIa in T1D and phase IIb in T2D
ORA2	BOWS Pharmaceuticals AG (Zug, Switzerland)	Capsule containing insulin in dextran matrix	Phase II in T2D; agreement with Orin Pharmaceuticals AG (Stockholm, Sweden) for the development
-	Emisphere Technologies (Cedar Knolls, NJ)	Eligen®; capsule containing insulin and an absorption enhancer that facilitates the passive transcellular transport	Phase II in T2D suspended
NN1952	Novo Nordisk (Bagsvaerd, Denmark)	GIPET® from Merrion Pharmaceuticals (Dublin, Ireland); capsule or tablet containing absorption enhancers that activate micelle formation, facilitating transport of insulin	Cancelled after phase II
NN1953; NN1954	Novo Nordisk (Bagsvaerd, Denmark)	Tablet of long-acting insulin analog	Phase I in T1D and T2D
IN-105	Biocon (Bangalore, India)	Insulin modified with a small PEG	Phase II: searching for other company to pursue development

HDV-I	Diasome (Conshohocken, PA)	Liposomal insulin, which is hepatic-directed vesicles-insulin, HDV-I, in orally administered forms	Phase III
-	Biolaxy (Shanghai, China)	NOD Technology; insulin-loaded bioadhesive nanoparticles	Phase I
-	Access Pharmaceuticals (Dallas, TX)	CobaCyte ™; nanoparticle or polymer containing insulin, coated with vitamin B_{12} for targeted delivery	Phase I

Table 2 – Oral Insulin Delivery Systems Undergoing Clinical Trials (Fonte et al, 2013)

Oramed Pharmaceuticals is developing an oral insulin product that consists of unmodified recombinant human insulin combined with adjuvants that protect it from enzymatic degradation in the gastrointestinal tract and promote its absorption from the gut. The aim of this study was to determine the optimal adjuvants to insulin ratio that can provide for the best pharmacodynamic profile, while maintaining the safety of the product. A decreased risk of hypoglycaemia has been observed in numerous studies where insulin was either administered directly to the portal vein or indirectly by way of peritoneal insulin administration or through peritoneal dialysates. (Eldor et al, 2010)

According with Medpage Today, an online medical news, a developer from Oramed announced in January 31, 2014 that a pill formulation of insulin has met safety and pharmacokinetic endpoints in a phase IIa trial. (Fiore, 2014) The compound, ORMD-0801, met its primary endpoint of safety and tolerability, as well as secondary endpoints of pharmacodynamics and pharmacokinetics. Oramed did not release numerical data, but said the full results would be presented at a scientific conference in the near future.

In April Oramed admitted that in a 6 weeks trial in 30 patients a "formulation issue resulted in diminished and inconsistent release of study drug." So a third of the patients - those designed to receive the higher doses of the drug at 24 mg - were compromised as far as the study was concerned, receiving only 8 mg of the drug. Despite this obvious setback, the study did highlight some of the drug's potential. For the uncompromised group who received the 16-mg dose, the patients showed a mean reduction in night-time glucose levels of about 23 mg/dL for the week compared with a placebo. And the fasting session from 5 a.m. to 7 a.m. provided a greater reduction of over 30 mg/dL on average. Three patients in

the group reported adverse events, which Oramed states were not related to the drug. (Gibney, April 30, 2014)

However, limited information is available so no conclusions can be made regarding the toxicity profiles of the different formulations and their components. Although, many of the components of the various nanoparticle formulations are included in approved oral drug products, as indicated by their listing in the Physician's Desk Reference and the U.S. FDA Inactive Ingredient Database.

At the present time information available in the published literature on the oral safety of food-related nanomaterials is lacking in terms of both quantity and quality.

It is clear that assessment of safety aspects of oral insulin dosing via nanoparticle-mediated systems has not received as much attention as has the assessment of efficacy. This is not surprising, given that formal toxicology testing is not expected to be initiated until a suitable nanoparticle formulation for the oral delivery of insulin has been identified and demonstrated to be effective in relevant animal models. (Card & Magnuson, 2011)

A lack of adequate physicochemical characterization places a limit on the value and significance of the results of a given study and makes it difficult, if not impossible, to compare studies and identify parameters that might influence efficacy and/or safety.

6 Discussion

Oral delivery of insulin is the most physiological way to replace the invasive parenteral route as well as a very promising area for research. The strategy for development of oral insulin has always been challenged for the researchers due to their high molecular weight, chemical or enzymatic degradation susceptibility, and impermeability through the intestinal mucosa. The high molecular weight of this class of drugs coupled with their hydrophilic nature restricts their transcellular permeation perhaps the most difficult hurdle to overcome. Nanotechnology offers various efficient carriers for the delivery of proteins, namely solid lipid nanoparticles, nano-structured lipid carrier, liposomes, niosomes, cubosomes and polymeric nanoparticles.

Nanoparticles could be identified as foreign substances by the immune system, causing the cells to react against their surface and the contents. This reaction can result in an inflammatory response by the body.

However, with all the benefits provided by nanotechnology, one has to look at the safety and toxicity of the nanoparticles that are being inserted into the bloodstream.

The efficacy of oral delivery of insulin is often limited because of their long-term efficacy and safety concerns need to be demonstrated through adequately powered studies in different patient populations across the diabetes spectrum. Furthermore, a reproducible absorption of insulin and understanding of meal-related absorption are also important goals for developing drug delivery systems that needs to be administered lifelong.

Clinical studies need to clearly demonstrate superiority of insulin nanoparticles over parenteral insulin formulations and oral hypoglycemic agents, including improved antihyperglycemic profile, reduced weight gain, and better disease progression outcome in long-term studies. The toxicological profile of the developed delivery systems must be also properly assessed.

7 Conclusions

In the last few decades, many research studies focused on the development of oral insulin delivery systems, able to circumvent the obstacles presented by the GIT enabling suitable insulin.

Microbiota influences the immune system through their ability to affect immune responses to pathogens and commensals, and most likely also autoimmunity response, since the microbial symbiotic colonization of the GIT may present the risk if epithelial or immune homeostasis is disturbed. Since insulin is a product which is subjected to autoimmune destruction, intestinal microbiome alterations has a particular role in autoimmune disease development. Prevention strategies based on the manipulation of the gut immune system may provide new paths for a cure to autoimmune diabetes. Mucus penetrating particles have the capability to improve oral drug delivery by penetrating in zones with a decreased mucus barrier being retained longer in the firmly adherent layer increasing distribution over the epithelium leading to a more effective treatment.

Some problems have not yet been overcome and extensive clinical trials are still needed. Many promising studies have been completed with various drugs. The vast array of in vitro systems and animal models that have been used, has produced discordant results regarding the optimum characteristics for efficient nanoparticles delivery in the GIT. Fewer data are available concerning the safety of these systems once they interfere the physicochemical and pharmacodynamics properties of the gastrointestinal mucosa.

Successful nano-carriers for the oral delivery of insulin must be able to increase insulin bioavailability to therapeutic levels, with minimal interindividual variability achieving with lower doses the desired hypoglycaemic effect.

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